STUDIES IN CHROMOSOME STRUCTURE AND REPLICATION

by

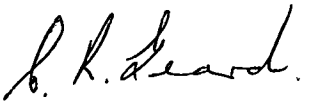
Charles Ray Geard.
B.Agr.Sc. (Melb.)

submitted in fulfilment of the
requirements for the Degree of
Master of Science

UNIVERSITY OF TASMANIA
HOBART

March, 1969

This thesis contains no material which has been accepted for the award of any other degree or diploma in any University and, to the best of my knowledge and belief, it contains no material previously published or written by another person, except when due reference is made in the text.



C.R. Geard

ACKNOWLEDGEMENTS

This study was initiated during the course of a Plant Breeding Fellowship from the Department of Agriculture, Hobart, Tasmania, in the Genetics section, Division of Plant Industry, C.S.I.R.O., Canberra, A.C.T., and continued in the Department of Botany, University of Tasmania.

Dr. W.J. Peacock, Principal Research Officer, Division of Plant Industry, C.S.I.R.O., Canberra, guided and inspired the outlook which made this and further studies possible. The advice and encouragement of Professor W.D. Jackson is gratefully acknowledged.

CONTENTS

SUMMARY

A quantitative analysis of sister chromatid exchanges and labelling patterns in H^3 thymidine labelled second division (post isotope incorporation) chromosomes of *Vicia faba* has demonstrated that:

(1) The frequency of sister chromatid exchanges per chromosome is proportional to chromosome length.

(2) The time of availability of colchicine may have a slight effect on the prospects of sister chromatid exchange events. However, overall, colchicine has no significant effect on the frequency of sister chromatid exchanges; on the incidence of iso-labelling or on the type of iso-labelling.

(3) The distribution of sister chromatid exchanges per chromosome follows a Poissonian form. The prospects of sister chromatid exchange events at either the first or second interphases are equal and independent.

(4) The ratio of single to twin sister chromatid exchange is consistent with the expectation that; sister chromatid exchanges occur independently of the tritium dose, and that chromosomal sub-units rejoin non-randomly, i.e. they exhibit polarity and are dissimilar in an analagous manner to the DNA double helix.

(5) A high frequency of chromosomes show isolabelling, the probability of which increases with chromosome length and/or exchange frequency.

(6) Twin and single isolabelling segments have been noted, however the ratios obtained do not fit those for sister chromatid exchanges and the origin of isolabelling remains unexplained.

Mitotic cycle determinations using the labelled mitosis wave method and the double labelling method have been carried out for *Vicia faba* and *Spironema fragrans* root tips. The variability of such methods has been commented on.

INTRODUCTION

Within the cell, chromosomes are products of molecular organisation concerned with the storage, transcription and replication of genetic information. That the chromosomes of higher organisms carry the factors of heredity has been known for many years (Wilson, 1925 pp.923 *et seq*, pp.1112 *et seq*; Darlington, 1960).

In order to achieve their functions within the cell, the chromosomes undergo structural changes which can vary consistently in time.

In their less condensed form i.e., in interphase, transcription and/or replication are carried out, the chromosomes being metabolically active; whereas in a condensed form, as in metaphase, the chromosomes are concerned with preservation and segregation. These varied morphological transitions and their observed size and structural complexities tend to obscure the fundamental organisation of the chromosome.

Studies of the distribution and behaviour of the chromosomes component molecules have been achieved in at least four technical means (see for instance Lewis and John, 1963):

(i) the chemical analysis of chromosomes;

(ii) the cytophotometric study of intact cells and/or individual chromosomes;

(iii) the study of enzymic degradation of chromosomes;

(iv) the autoradiographic study of isotopically labelled chromosomes.

Either light or electron microscopy can be used with (iii) and (iv).

This study presents results from experiments utilising autoradiography in conjunction with light microscopy.

The chemical analysis of chromosomes has primarily involved the analysis of isolated interphase nuclei. Miescher in 1871 (cited by Wilson, 1925, p.640) carried out the first important investigation of the chemical nature of the cell nucleus. He isolated a complex nucleoprotein from salmon spermatazoa and found similar if not identical substances in sperm heads of other species, as well as in nuclei of certain somatic cells. The whole complex he termed "nuclein" while an organic base component which was present in considerable quantities in fish sperm he termed "protamin". Kossel in 1884 (cited by Stedman and Stedman, 1947, p.234) in a study with goose erythrocytes found a more complex basic protein in combination with the nucleic acid, and introduced the name "histone".

Since fish sperm possesses the composition claimed it follows that nucleic acid is a major component of the chromosomes. Cytological characterisation of chromosomes is at times based on the nucleic acids and their basophilic staining properties.

Further chemical analyses in a comprehensive range of higher organisms have been carried out. Stedman and Stedman (1947) found that all nuclei contained nucleic acid and basic proteins and also acidic proteins, which they termed "chromosomin". They postulated that "chromosomin" was the hereditary material since they believed that proteins were the only compounds capable of accounting in a broad manner for the hereditary functions of the chromosomes. Mirsky (1947) found desoxyribonucleic acid (DNA), ribonucleic acid (RNA), either histones or protamines and some non-histone protein in salmon erythrocytes and sperm, liver, kidney and salivary gland nuclei. Between tissues from the one organism he found profound differences in RNA and non-histone protein content but not for DNA content.

These earlier analyses on isolated nuclei have recently been confirmed by the analysis of isolated metaphase chromosomes (Maio and Schildkraut, 1967; and Huberman and Attardi, 1966). Their results show that DNA, RNA and both acid soluble and acid insoluble proteins are

associated in the chromosomes. Maio and Schildkraut (1967) believe that ribosomal RNA was associated with the chromosomes since they found that over eighty per cent of the extracted RNA was similar to ribosomal RNA.

Therefore, it is probable that a DNA - residual protein complex is responsible for maintaining chromosome structure. It does not appear possible at this stage to confine responsibility for basic chromosome structure to any of the molecular components, since there is considerable difficulty in relating information at the molecular level to the structure of the chromosome itself.

Prescott (1961) has commented on the tendency to relegate the protein content of chromosomes to a position of minimal importance. Busch *et al* (1964) are doubtful that histones are related to the structure of metaphase chromosomes, but they believe that other acidic nuclear proteins may be. Presumably the different morphological states of the chromosome are the result of varying protein configurations; and protein synthesis is an essential part of chromosomal duplication. However chromosome duplication is sometimes tacitly equated with DNA synthesis, and this is the case with this study, since only the DNA component of the chromosomes will be considered throughout.

The work leading to the discovery that DNA is the hereditary material and the implications therefrom are well documented in many texts e.g. Watson (1965); Stahl (1964); Sager and Ryan (1961). The Watson-Crick-Wilkins helical structure for DNA (Watson and Crick 1953a., b., c., Wilkins, Stokes and Wilson, 1953) changed the whole concept of nucleic acid as being a "molecular midwife" (Darlington, 1947 p.266) to that of being a "molecular mother".

Chromosome studies utilising autoradiography.

Thymidine is a specific precursor for DNA (Reichard and Eastborn, 1951); therefore if thymidine is "labelled" in some way and made available to cells it will be taken up and be apparent only in the chromosomes. This is possible using the technique of autoradiography (for applications in biology and problems, see Taylor, 1956; Ficq, 1959; Perry, 1965). This technique involves the recording of ionising particles from a radio-isotope in a special photographic emulsion super-imposed on the material containing the isotope.

For autoradiographic studies at the chromosomal level tritium allows a very precise localisation because its emitted beta particles are of low energy (approximately 0.018 Mev) and affect only one silver grain in a photographic emulsion (Ficq 1959); giving a resolution of

± 1 μ and depending on the preparation possibly less (Taylor, 1956; Perry, 1965 Table II p.311). Taylor (1956 p.573) stated, "Although tritium has not been used much in autoradiography, it should prove very useful for high resolution work. With its long half life and low energy beta particles it should be a good label for chromosomes that are to be followed through several divisions."

Taylor thoroughly substantiated this statement when he used the combination of thymidine and tritium in a series of autoradiographic studies with chromosomes which Stahl (1964 p.70) has referred to as, "the most compelling experiments to date on the organisation of DNA in chromosomes". (see Taylor, Woods and Hughes, 1957; Taylor, 1958, 1959).

The unit of observation was not the DNA molecule but the DNA contained in a chromosome, the study of which enabled conclusions to be reached on the molecular organisation of DNA within the chromosome.

Experimental evidence on the behaviour of DNA in chromosomes

Taylor, Woods and Hughes (1957) grew broad bean (*Vicia faba*) roots in a solution containing tritiated thymidine for a time. They then followed the course of the tritiated thymidine in the chromosomes over two cellular divisions. They found that at the first division

following isotope incorporation, label was equally distributed over both chromatids of each metaphase chromosome. At the following division label was confined to one chromatid, except in cases where only part of one chromatid was labelled opposite its partners unlabelled region. These reciprocal events were termed "sister chromatid exchanges".

Taylor *et al* (1957) were able to assign cells to their appropriate division since after labelling the material was transferred for further growth to a medium containing colchicine, which permits chromosome duplication while preventing cytoplasmic division. Therefore cells with twenty four chromosomes (tetraploids) were second division post labelling cells.

The results obtained demonstrated that the anaphase chromatid consists of two DNA sub-units both of which are replicated and segregated from each other at the first division; resulting in equal labelling of the daughter chromatids which are themselves double. This doubleness is shown at the second division when the old unlabelled and new labelled sub-units segregate from each other. This study complemented a parallel investigation on isotope incorporation into the DNA of rapidly dividing *Escherichia coli* cells by Meselson and Stahl (1958).

The Watson-Crick theory of semi-conservative DNA duplication was confirmed at the molecular level (Meselson and Stahl, 1958) and paralleled at the chromosomal level (Taylor *et al*, 1957). Rightly so, these two experiments are well documented in biology texts e.g., Kimball (1965 pp.457-459).

The semi-conservative replication of DNA in higher organisms has been confirmed by Simon (1961) in mammalian cells and Filner (1965) and Haut and Taylor (1967) in plant cells; using density gradient centrifugation similar to the technique of Meselson and Stahl (1958).

Autoradiographic experiments have with some exceptions also confirmed the general pattern of isotope distribution shown by Taylor *et al* (1957), (see Taylor, 1963 p.76).

Exceptions to the general pattern

La Cour and Pelc (1958) observed in *Vicia faba* that radio-activity as determined in grain development was *not* equally distributed over both chromatids at the first division if colchicine was present during labelling. If colchicine was omitted they found that sister chromatids at the second division were in some instances labelled in the same region. They concluded that the results of Taylor *et al* (1957) were inconclusive in that the colchicine used may have influenced the distribution of

the newly synthesised DNA. Woods and Schairer (1959) questioned these observations and presented data of grain counts over sister chromatids at the first division after labelling; showing no significant differences either with or without colchicine. Peacock (1965) has also carried out statistical tests of grain distribution and shown no significant asymmetry between chromatids at the first division. However Taylor (1963) has found that the use of high specific activity tritiated thymidine produces an apparent significant asymmetry (as observed by La Cour and Pelc, 1958) which he interprets as "evidence for the out of phase replication of the two sub-units of a chromatid", (Taylor 1963 p.76).

La Cour and Pelc (1959) replied to the criticism of Woods and Schairer (1959) by pointing out differences in their respective experimental methods and stressed the occurrence of label over equivalent areas of second division sister chromatids. This was also found in rare instances by Taylor (1958) but he considered (Taylor 1963 p.76) that such anomalies, "could result from exchanges among the four chromatids which were the descendants of a pair of labelled chromatids. Colchicine which probably keeps such pairs together, would be expected to increase the frequency of this event".

Peacock (1963) varied the time of colchicine treatments in autoradiographic experiments with *Vicia faba*. He obtained consistent results between treatments for both first and second division scored chromosomes. Therefore the claim of La Cour and Pelc (1958) that colchicine influences the distribution of newly symthesised DNA was not supported. He also found from a quantitative analysis of sister chromatid exchanges that colchicine had no influence on the prospects of an exchange event. However in each experiment he found a significant proportion of second division chromsomes were labelled over similar regions of both chromatids i.e., "isolabelled". Since "isolabelling" cannot readily be accommodated on the basis of a single DNA molecule per chromatid Peacock (1963) interpreted his results to accommodate exchanges between sub-chromatid units and concluded that *Vicia faba* chromosomes could have a lateral multiplicity of DNA molecules.

Other reports of isolabelling have been by Gay (1965) in *Haplopappus gracilis*, Walen (1965) in cultured cells of the marsupial *Potorous tridactylis* and Evans (personal communication to Peacock, 1965) in *Allium*.

Zweidler (1964) also working with *Allium* has reported different results. After determining the mitotic cycle of *Allium cepa* he carried out autoradiographic experiments

where metaphase cells were allocated to their respective divisions by the time at which they were found post labelling. On this basis he has reported that at first division both chromatids of each chromasome were strongly labelled; at the second division both chromatids were labelled but not as much; at the third division one chromatid was labelled and the other not, except for sister chromatid exchanges; at the fourth division only part of one chromatid was labelled.

A few chromosomes at the second division were observed with one chromatid partially unlabelled, while a few chromosomes at the third division showed isolabelling. On the basis of these observations Zweidler (1964) has concluded that at least two replication units per chromatid must be present, with the first detectable separation taking place at the second division. Therefore he concludes that the chromosomes of *Allium cepa* are normally polytenic and are "kryptotetraploid" (Zweidler, 1964 p.62). Since Gall (personal communication to Taylor, 1963) found a regular semi-conservative behaviour of *Allium* chromosomes; and Evans (personal communication to Peacock, 1965) finds examples of isolabelling in second division chromosomes of *Allium*, Zweidler's results require confirmation.

Autoradiographic evidence on the organisation of DNA within chromosomes

Taylor (1958, 1959) used quantitative studies of sister chromatid exchanges to provide information on the organisation of chromosomal DNA. Studies on second division metaphase cells (tetraploids induced by colchicine) of *Bellevalia romana* allowed the classification of sister chromatid exchanges into two types. Twin exchanges were exchanges which occurred at identical places on two sister chromosomes and single exchanges were those where there was no corresponding paired exchange. Taylor (1958) suggested that chromosomes with twin exchanges derived from one original labelled chromosome; exchange between chromatids taking place at the first division after labelling.

The expectation of the ratio of twin to single sister chromatid exchanges of second division paired sister chromosomes depends on whether re-union of the chromatid subunits is restricted or unrestricted. If the chromatid sub-units were analagous to the two chains of the DNA molecule, they would be complementary and therefore re-union would be restricted. This is demonstrated in Figure 1. while the case which applies if the strands of the sister chromatids are alike and rejoin at random is shown in Figure 2.

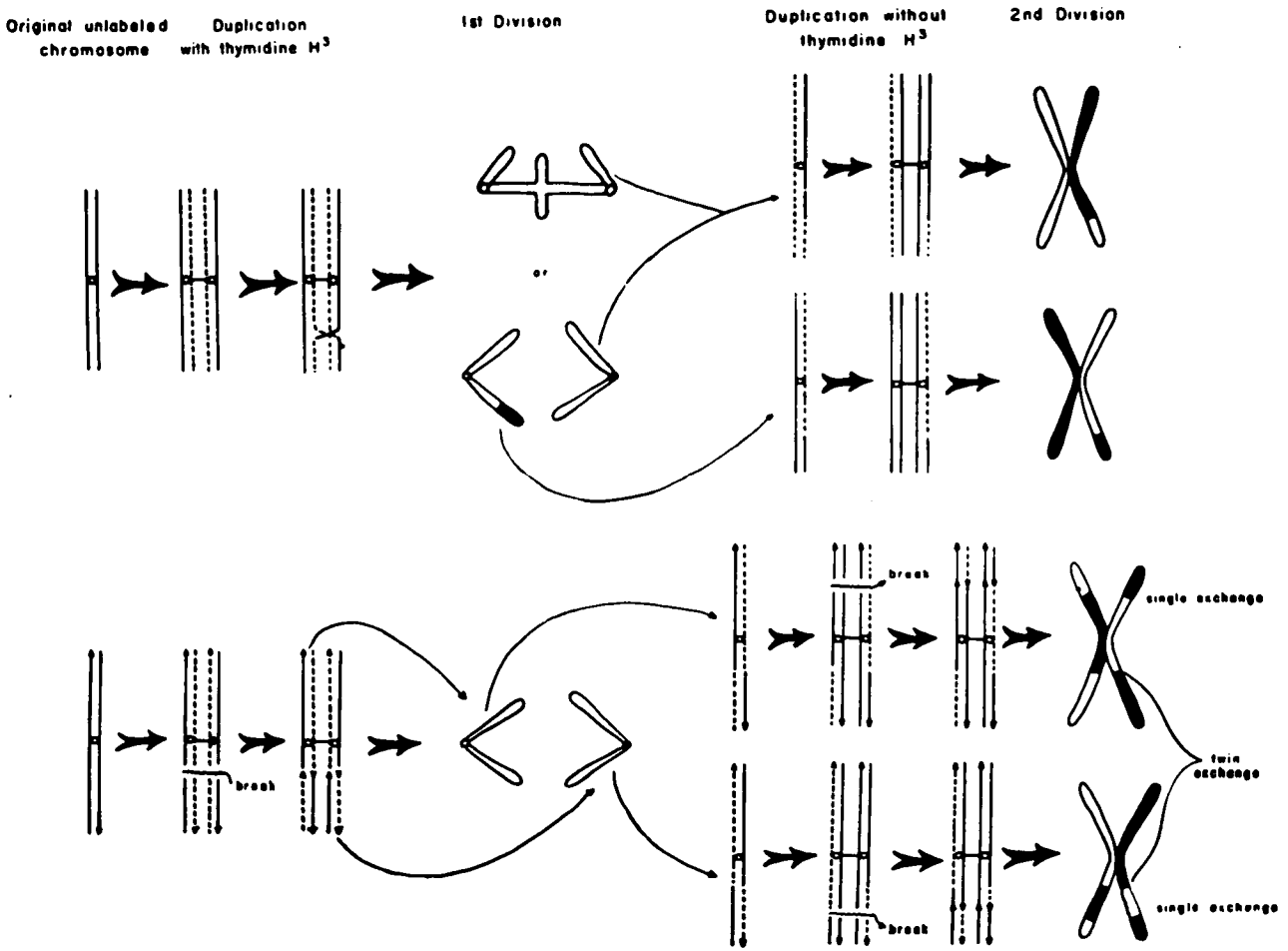


FIGURE 4. Schematic drawing to show the segregation and exchange of labeled parts of chromosomes after one duplication in thymidine-H^3. *Upper* diagram shows the results of exchanges between two of the four DNA sub-units of sister chromatids. The *lower* diagram shows the expected frequency of single and twin exchanges if a difference between the DNA sub-units exists. The difference is represented by arrows. The dashed lines and the regions of chromatids in outline represent labeled parts. Solid lines and regions in black represent unlabeled parts.

Figure 1. Direct from Taylor (1959).

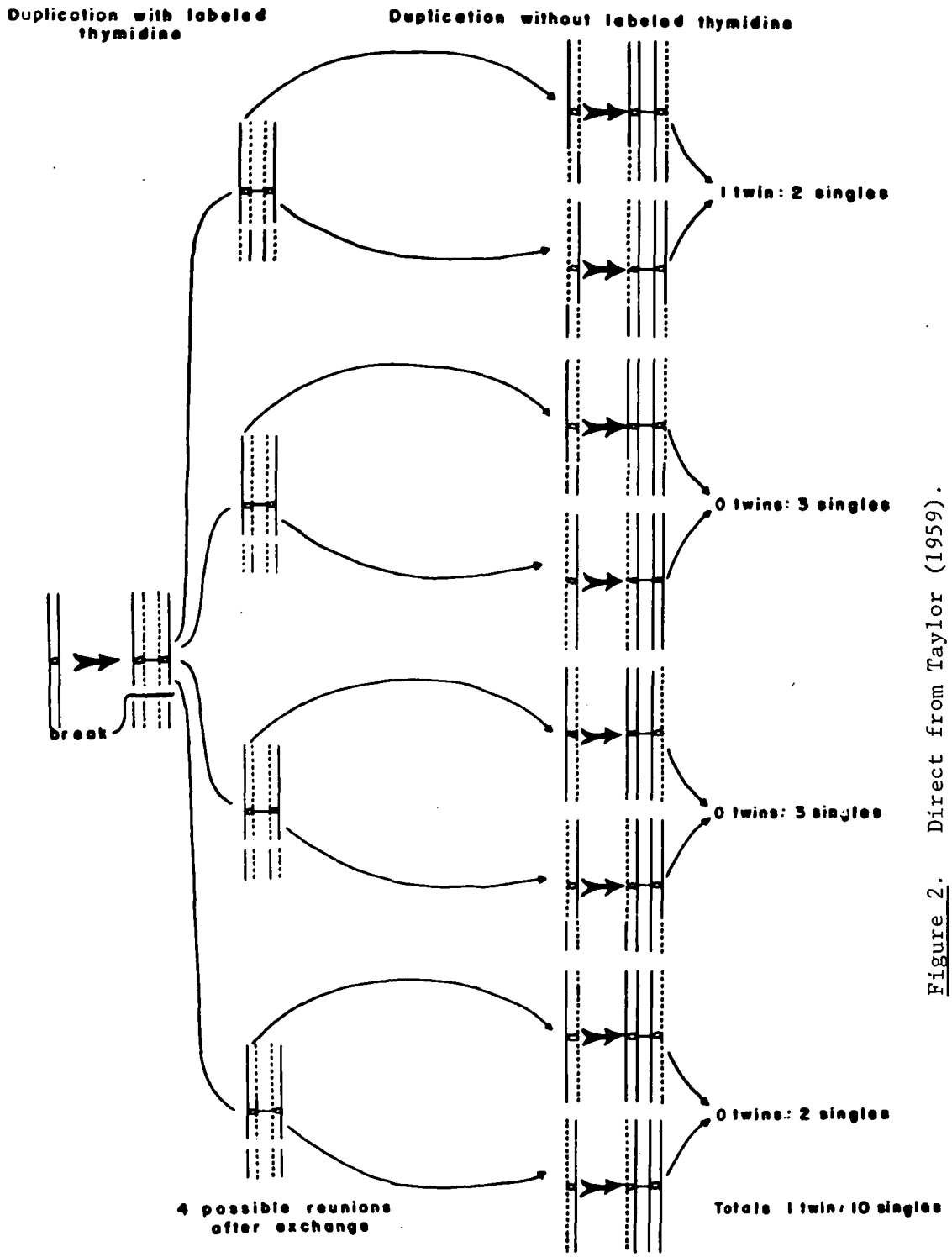


Figure 2. Direct from Taylor (1959).

FIGURE 5. Diagram to show the expected frequency of single and twin exchanges if the two sub-units of each chromatid are alike and are capable of reunion at random. Dashed lines represent labeled parts. Exchanges following the second duplication are not shown in detail, but each chromosome is assumed to have one exchange at the second interphase after or during duplication.

Taylor's predictions of:

2 single exchanges : 1 twin exchange for restriction of reunion and 10 single exchanges : 1 twin exchange for unrestricted reunion were based on the assumption that sister chromatid exchanges are spontaneous events. The first results obtained did not fit either prediction *viz.* 30 single exchanges : 80 twin exchanges (from Taylor 1958 p.521). However the ratio found (0.37 : 1) was closer to a 2 : 1 ratio than a 10 : 1 ratio, such that the no restriction hypothesis could be discarded.

Therefore Taylor concluded : "This frequency can be explained only on the hypothesis that the two strands of the chromosome are unlike i.e. are not free to re-unite at random. Therefore the chromosome has two features in common with the Watson-Crick model of DNA. It has two strands and the strands are different in some structural feature that restricts reunion to like strands when chromatid exchanges occur". (Taylor, 1958 p.528).

Although this conclusion was reasonable on the basis of the two predicted hypotheses, Taylor (1959) sought an explanation for the considerable divergence from either ratio. Colchicine was present during the second but not the first interphase, and the radioactivity per chromosome should have been twice as much during the first than the second interphase. Therefore colchicine was supplied at

the same time as the tritiated thymidine, and since a lag might have occurred in the colchicine effect, it was also supplied two hours before the tritiated thymidine. The results were (Taylor, 1959 p.70):

viz. colchicine with tritiated thymidine

36 single exchanges : 30 twin exchanges;

colchicine before tritiated thymidine

26 single exchanges : 14 twin exchanges

Therefore the conclusions of Taylor (1958) were confirmed and also colchicine was shown to affect the prospect of sister chromatid exchanges in *Bellevalia romana*. The results obtained by Taylor *et al* (1957) and Taylor (1958, 1959) suggest that

(1) Each chromatid consists of two parts.

(2) Each of these two parts remains intact during duplication.

(3) The two parts are dissimilar and complementary.

The simplest requirements of the Watson-Crick model for DNA are met and each chromatid could therefore consist of one or a linear series of DNA molecule(s).

Exceptions to the labelling patterns found by Taylor *et al* (1957) have been presented while Taylor's conclusions with regard to sub-unit dissimilarities are complexed by the unexplained effect of colchicine in *Bellevalia romana*.

Peacock (1963) considered that the karyotype of *Vicia faba* precluded a quantitative analysis of sister chromatid exchanges, however the large metacentric (M) chromosome is amenable to such an anlysis. Also Peacock (1963) has shown that there is no effect of colchicine on the prospect of sister chromatid exchanges in *Vicia faba*. Therefore this organism was chosen for a comprehensive quantitative analysis of sister chromatid exchanges.

The correct interpretation of results depends on a knowledge and understanding of the time relations of the various stages of the mitotic cycle. Therefore a preliminary requirement to further work is the determination of mitotic cycle times under the experimental conditions to be employed. This was carried out for *Vicia faba* and also for *Spironema fragrans* (see Section II).

SECTION I

Autoradiographic analysis of the chromosomes of *Vicia faba*

Materials and Methods

Secondary roots of *Vicia faba* (*var.* Cole's Dwarf Prolific) were cultured in fully aerated sterilised modified Hoagland's solution. Tritiated thymidine (Amersham) at the rate of 2 µc/ml with a specific activity of 3.0 Cu/mM was made available to the roots for two hours. At the termination of labelling the roots were washed thoroughly in running tap water and returned to an isotope-free aerated culture solution. All experiments were carried out at 22 ± 0.5°C. Four different colchicine treatments were used (colchicine concentration 0.025% in culture solution).

A 1 hour colchicine, 3 hours subsequent to removal of the isotope (3 replicate experiments)

B 1 hour colchicine during the second hour of isotope treatment (2 replicate experiments)

C 1 hour colchicine, 1 hour prior to isotope treatment (1 experiment)

D 1 hour colchicine 18 hours subsequent to removal of the isotope (1 experiment).

The times of fixation of root tips varied slightly from experiment to experiment. Four to six root tips being taken at each fixation time.

Fixation times	AI	18-24	hours	post	label	1/2 hourly
	II	21-29	"	"	"	" "
	III	19-26	"	"	"	" "
	BI	19-26	"	"	"	" "
	II	22-29	"	"	"	hourly
	C	22-29	"	"	"	"
	D	19-26	"	"	"	1/2 hourly

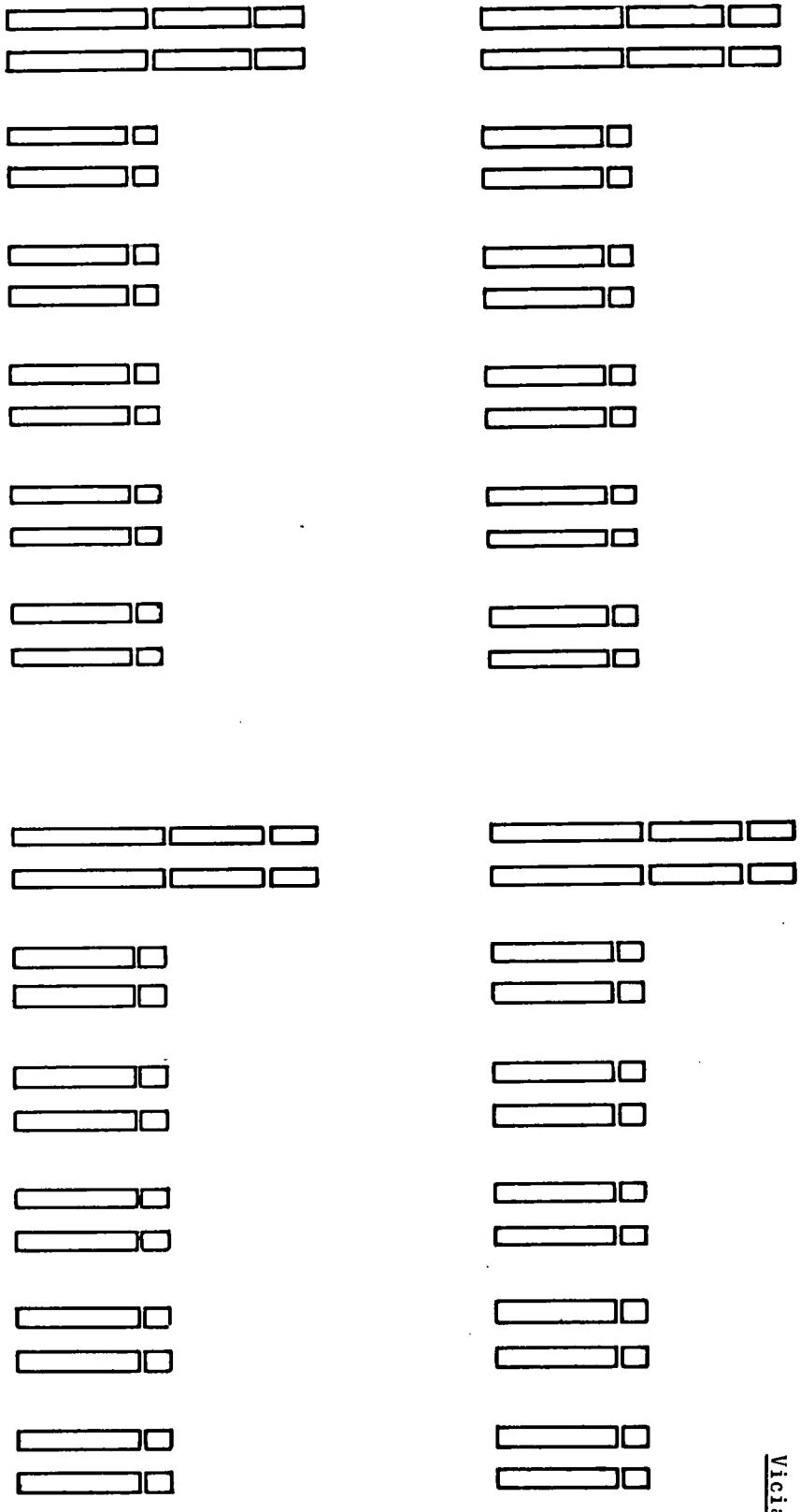
Fixation was in ethyl alcohol : acetic acid 3 : 1 for at least two hours under refrigeration. Root tips were hydrolysed in N HCl for 10 minutes at 60°C and stained by the Feulgen technique. Squashes of the terminal heavily stained portion of the meristem were made on subbed slides in 45% acetic acid after removal of the root cap. Cover slips were removed after freezing in liquid air or on solid carbon dioxide, and the slides placed in absolute alcohol. Slides were then taken through the alcohol series (5 minutes in each of 95%, 90%, 70%, 50% and 30% ethyl alcohol in water) to distilled water.

Prior to dipping in Kodak NTB or Ilford K2 nuclear track emulsion the slides were brought to 40°C in distilled water. Slides were dipped in emulsion at 40°C in the dark room with sole illumination through a Kodak

Wratten series 2 safelight filter. Slides were allowed to dry then placed in light tight boxes containing silica gel and kept under refrigeration. One or two slides were developed and examined for suitable grain density at intervals until all slides were developed at varying times from 4 to 12 weeks post dipping. Autoradiogram development was carried out in Kodak Dektol : distilled water 1 : 1, for 2 minutes at approximately 18°C; rinsed in distilled water; fixed in Kodak acid fixer for 5 minutes and finally washed in running water for at least 30 minutes. Slides were allowed to dry and cover slips mounted in Euparal.

All slides were scanned under low power. Those showing a reasonable incidence of labelled tetraploid cells (except for treatment D) were retained for extensive scanning and scoring under oil immersion. Each suitably labelled metaphase or colchicine anaphase cell was considered separately. The karyotype of *Vicia faba* consists of one metacentric (M) and five subelocentric (S) chromosomes; the S chromosomes being individually indistinguishable.

The pattern of labelling over the chromosomes was drawn on sheets illustrating an idealised karyotype of *Vicia faba* (Figure 3). In treatments A, B and C only chromosomes in definite tetraploid cells were scored. The



Vicia faba

Figure 3. Idealised karyotype of *Vicia faba*.

frequency of sister chromatid exchanges and the frequency and class of isolabelling (Peacock, 1963) in both S and M chromosomes were recorded in all experiments. Only fully labelled chromosomes were scored for sister chromatid exchanges.

Twin and single exchanges were scored in pairs of sister M chromosomes. Only those tetraploid cells (in A, B and C) in which 3 or all 4 of the metacentrics could be scored were included in the analysis. Two hours label is adequate to completely label chromosomes, however many cells are found with discontinuously labelled chromosomes. Asynchromy of replication of the 2 original M chromosomes produces 2 pairs of dissimilarly labelled M chromosomes at the second division metaphase. Similar discrete regions of label on chromosomes enable sister pairs to be recognised; therefore discontinuous label is an advantage for this analysis. In many instances it was felt that pairing on this basis was unequivocal, while in the remaining cases the position of exchanges assisted in deciding pairs, providing possibly ambiguous pairs of chromosomes.

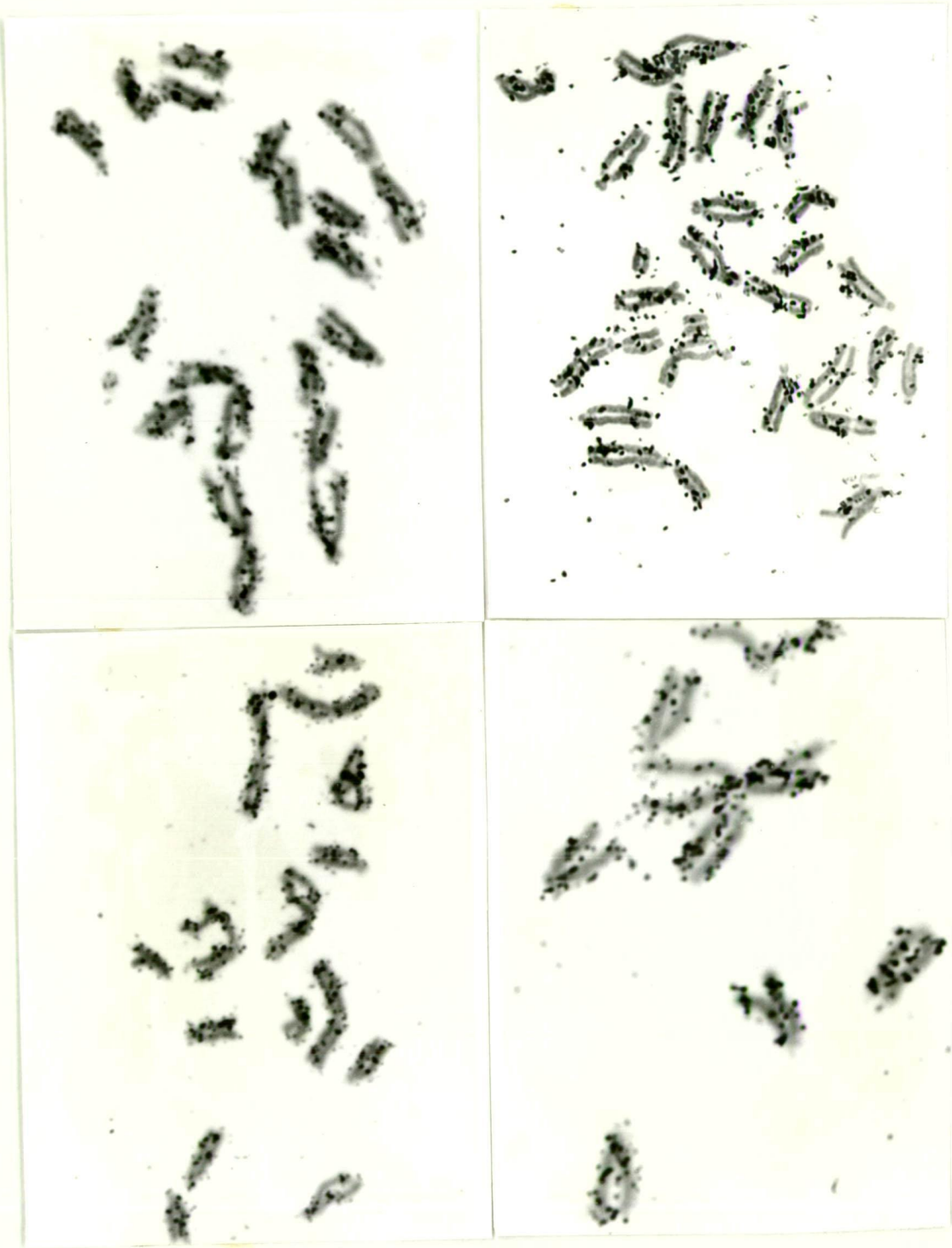


Figure 4. Various metaphase chromosome configurations of *Vicia faba* depicting second division labelling patterns.

Results

In all experiments the slides with the greatest number of labelled second division metaphases were found from fixations 24 to 27 hours. In treatments A, B and C these could be identified by tetraploidy as well as label distribution patterns. Diploid cells of similar pattern were found over this period in treatment D. On the basis of the mitotic cycle determinations in Section II, there has been little or no radiation induced delay in these experiments (*cf.* Peacock, 1963).

For the majority of parameters that were scored, replicate experiments were homogeneous, and therefore results have been bulked for comparisons between treatments. The results obtained for replicate experiments are recorded in Appendix I.

a. Distribution of label in second division chromosomes

In all experiments there was a consistent higher incidence of isolabelling than has been reported previously. When scoring, the labelling pattern was depicted as accurately as possible on the idealised karyotype sheets. Where labelling occurred on identical regions of both chromatids a chromosome was counted as being isolabelled. Tables 1 and 2 record the incidence of isolabelling on S and M chromosomes respectively.

Table 1.

Label distribution in S chromosomes

Treatment	Chromosomes with label segregation	Chromosomes with isolabelling	Total chromosomes scored	% isolabelling
A	324	250	574	43.6
B	228	189	417	45.3
C	71	54	125	43.2
D	191	135	326	41.4
Total	814	628	1442	43.6

N.B. Replicates from treatment B were heterogeneous

Between treatments $\chi^2_{3\ d.f.}$ 1.15 0.70>P>0.50

Within treatments $\chi^2_{3\ d.f.}$ 9.20 0.05>P>0.02

Table 2.

Label distribution in M chromosomes

Treatment	Chromosomes with label segregation	Chromosomes with isolabelling	Total chromosomes scored	% isolabelling
A	56	126	182	69.2
B	26	85	111	76.6
C	16	45	61	73.8
D	22	44	66	66.7
Total	120	300	420	71.4

Between treatments $\chi^2_{3\ d.f.}$ 2.77 0.50>P>0.30

The heterogeneity χ^2 test between treatments (Tables 1 and 2) show that the time of application of colchicine has no influence on the proportion of isolabelling. However incidence of isolabelling increases with chromosome length.

b. Chromosomes showing label segregation

In order to analyse the frequency of sister chromatid exchange events those chromosomes without any isolabelling will be considered separately. For the purposes of analysis class frequencies have been summed to that class with an expectation of 5 or greater (Mather, 1966 p.175).

Table 3.

Frequency of sister chromatid exchanges in S Chromosomes

Treatment	No. of exchanges per chromosome							Total chromosomes scored
	0	1	2	3	4	5	6	
A	55	111	107	39	9	3	0	324
B	32	63	72	43	13	4	1	228
C	7	24	22	15	3	0	0	71
D	21	78	60	23	7	1	1	191
Totals	115	276	261	120	32	8	2	814

Between treatments χ^2_{12} d.f. 21.51 $0.05>P>0.02$

Table 4.

Frequency of sister chromatid exchanges in M chromosomes

Treatment	No. of exchanges per chromosome											Total chromosomes scored
	0	1	2	3	4	5	6	7	8	9	10	
A	1	1	6	13	11	15	5	3	1	0	0	56
B	0	2	4	6	5	3	5	0	1	0	0	26
C	0	0	1	5	2	5	2	1	0	0	0	16
D	0	3	2	6	4	2	3	1	0	0	1	22
Total	1	6	13	30	22	25	15	5	2	0	1	120

χ^2 test not applicable.

Table 5.

Mean number of sister chromatid exchanges per chromosome
(standard error of the mean in brackets)

Treatment	S chromosomes	M chromosomes	Ratio S : M
A	1.52 (0.06)	4.11 (0.21)	1 : 2.7
B	1.82 (0.08)	3.88 (0.35)	1 : 2.1
C	1.76 (0.12)	4.31 (0.35)	1 : 2.4
D	1.60 (0.08)	3.91 (0.47)	1 : 2.4
Over all treatments	1.64 (0.04)	4.05 (0.16)	1 : 2.5

From Tables 3 and 5 there is an indication that time of application of colchichine has a slight effect on sister chromatid exchanges in S chromosomes but not in M chromosomes (Table 5). Since the direct χ^2 test on the observed class frequencies involves the grouping of terminal classes the level of significance obtained is not adequate for a definite conclusion to be made. However if colchicine is present during the first S and G1 periods, sister chromatid exchanges appear to be increased.

The relative lengths of the S and M chromosomes were derived from the work of Martin and Shanks (1966). The S chromosomes vary slightly in length; the ratio of S : M chromosomes being 1 : 2.4. This is in good agreement with the ratio of exchanges for S and M chromosomes, and shows that exchange frequencies are proportional to chromosome length.

If sister chromatid exchanges are produced by statistically independent events the frequency distribution of chromosomes in sister chromatid exchange classes should follow a Poissonian form. Using the determined mean the expected terms to fit the Poisson distribution can be found (Pearson and Hartley, 1954 Tables 7 and 39). The observed : expected differences can then be tested using χ^2. Since means differ for sister chromatid exchanges per S chromosome between

treatments; treatment totals are tested against a Poisson distribution; (Table 6) and for M chromosomes the overall total frequency distribution is tested against a Poissonian form (Table 7).

In the Poisson distribution the mean and the variance are equal. The variance/mean ratio therefore provides a test of the Poissonian nature of a distribution, avoiding any grouping of terminal classes, and preserving any trends in the deviations from expectation (as used by Jackson and Barber, 1958 Tables 6 and 7). The significance of deviations of the ratio from unity can be tested by using the ratio of the sum of squares to the mean, which has a χ^2 distribution (Anderson and Bancroft, 1952 pp.139 *et seq*). For some distributions numbers were inadequate for test against a Poisson, and the variance/mean ratio for all individual distributions are given in Appendix I.

From Table 6, for two treatments the distributions differ significantly from a Poisson. It can be seen from the tables that there is some under-dispersion. This is substantiated by the variance : mean ratios from individual experiments (see Appendix I).

c. Total chromosomes

If results from those chromosomes with isolabelling are added to that of chromosomes showing label segregation

Table 6.

S. chromosomes. Distribution in sister chromatid exchange categories fitted by Poisson

Treatment		Number of exchanges per chromosome						Number of chromosomes	χ^2	d.f.	P
		0	1	2	3	4	≥5				
A	Data	55	111	107	39	9	3	324			
	Poisson	70.9	107.7	81.9	41.5	15.8	6.3		16.17	4	xx
B	Data	32	63	72	43	13	5	228			
	Poisson	36.9	67.2	61.2	37.1	16.1	9.5		6.11	4	0.20-0.10
C	Data	7	24	22	15	3	0	71			
	Poisson	12.2	21.5	18.9	11.1	4.9	2.4		6.92	3	0.10-0.05
D	Data	21	78	60	23	7	2	191			
	Poisson	38.6	61.7	49.4	26.3	10.5	4.5		17.42	3	xxx

x significant at 0.05 level, xx significant at 0.01 level, xxx significant at 0.001 level.

Table 7.

M chromosomes. Distribution in sister chromatid exchange categories fitted by Poisson

		Number of exchanges per chromosome										Total number of chromosomes	χ^2	d.f.	P
		0	1	2	3	4	5	6	7	8	≥9				
All treatments	Data	1	6	13	30	22	25	15	5	2	1	120			
	Poisson	2.1	8.5	17.1	23.2	23.4	19.0	12.8	7.4	3.8	2.7		9.22	6	0.20-0.10

similar tables can be formed. If it is assumed that isolabelling represents regulation following an exchange event (Peacock, 1963) then the class of isolabelling found can be equated with exchanges. In this study, terminal and complete isolabelling are taken as equivalent to 1 exchange (*contra* Peacock, 1963 who considered complete isolabelling as equal to no exchange) and intercalary isolabelling as equivalent to 2 exchanges. For M chromosomes in particular, isolabelling was frequently found in combination with sister chromatid exchanges. For both S and M chromosomes the overall total frequency distributions are tested against a Poissonian form (Tables 11 and 12).

The tests show that the distributions of total exchanges per chromosome are not Poisson distributions. In both cases the distributions are under-dispersed. Tables 8, 9 and 10 show the results of total exchanges for all chromosomes in all treatments.

Table 8.

Frequency of total exchanges in S chromosomes

Treatment	No. of exchanges per chromosome							Total chromosomes scored
	0	1	2	3	4	5	6	
A	55	223	175	81	27	13	0	574
B	32	143	130	86	21	4	1	417
C	7	40	45	24	9	0	0	125
D	21	131	108	49	14	2	1	326
Total	115	537	458	240	71	19	2	1442

Between treatments $\chi^2_{12\ d.f.}$ 16.52 $0.20>P>0.10$

Table 9.

Frequency of total exchanges in M chromosomes

Treatment	No. of exchanges per chromosome											Total chomosomes scored
	0	1	2	3	4	5	6	7	8	9	10	
A	1	8	13	35	34	38	26	19	5	3	0	182
B	0	4	6	20	26	25	17	8	3	1	1	111
C	0	0	2	20	11	11	9	4	1	3	0	61
D	0	3	6	19	14	9	7	5	1	1	1	66
Total	1	15	27	94	85	83	59	36	10	8	2	420

N.B. Replicates from treatment A were heterogeneous

Between treatments $\chi^2_{15\ d.f.}$ 14.27 $0.70 > P > 0.50$

Within treatments $\chi^2_{13\ d.f.}$ 31.94 $0.01 > P > 0.001$

Table 10.

Mean number of exchanges per chromosome
(including standard errors in brackets)

Treatment	S chromosomes	M chromosomes	Ratio S : M
A	1.72 (0.05)	4.51 (0.13)	1 : 2.6
B	1.85 (0.05)	4.56 (0.16)	1 : 2.5
C	1.90 (0.09)	4.59 (0.22)	1 : 2.4
D	1.74 (0.06)	4.20 (0.23)	1 : 2.4
Totals	1.78 (0.03)	4.49 (0.09)	1 : 2.5

The heterogeneity χ^2 test shows that there is no significant difference between treatments. As before the number of exchanges is higher if colchicine is available during first S and G1. Also, as before total exchange frequencies are proportional to chromosome length.

Table 11.

S chromosomes. Distribution in total exchange categories, fitted by Poisson

		Number of exchanges per chromosome						Total number of chromosomes	χ^2	d.f.	P
		0	1	2	3	4	≥ 5				
All treatments	Data	115	537	458	240	71	21	1,442			
	Poisson	244.2	433.2	384.8	233.0	96.6	51.1		131.59	4	xxx

Table 12.

M chromosomes. Distribution in total exchange categories, fitted by Poisson

		Number of exchanges per chromosome											Number of chromo-somes	χ^2	d.f.	P
		0	1	2	3	4	5	6	7	8	9	≥10				
All treatments	Data	1	15	27	94	85	83	59	36	10	8	2	420			
	Poisson	4.7	21.0	47.2	70.9	79.7	71.7	53.8	34.6	19.5	9.7	7.2		31.19	8	xxx

d. Classes of isolabelling

Chromosomes with isolabelling can be classified depending on whether they have terminal, complete or intercalary isolabelling. Metacentric chromosomes in particular, were observed with up to three regions of isolabelling along their length e.g. terminal isolabelling at each end and an intercalary region of isolabelling. The frequency of classes of isolabelling are given in Tables 13 and 14.

Table 13.

Frequency of classes of isolabelling on isolabelled S chromosomes

Treatment	Complete	Terminal	Intercalary	Total	Chromosomes with isolabelling
A	34	184	51	269	250
B	14	140	45	199	189
C	5	35	18	58	54
D	12	96	31	139	135
Totals	65	455	145	665	628

Between treatments $\chi^2_{6\ d.f.}$ 7.94 $0.30 > P > 0.20$

Table 14.

Frequency of classes of isolabelling on isolabelled M chromosomes

Treatment	Complete	Terminal	Intercalary	Total	Chromosomes with isolabelling
A	4	87	91	182	126
B	2	57	62	121	85
C	0	27	32	59	45
D	0	28	32	60	44
Totals	6	199	217	422	300

Between treatments $\chi^2_{3\ c.f.}$ 2.57 $0.50 > P > 0.30$

N.B. Completely isolabelled M chromosomes were included with terminals for χ^2 analysis. The contingency test indicates that colchicine has no influence on the formation of an isolabelled region.

e. Single and twin sister chromatid exchanges

Where 3 or 4 metacentric chomosomes were available for scoring in one tetraploid cell, sister chromosomes were paired on the basis of their label distribution patterns, in some cases assisted by their exchange distribution patterns. Sister chromatid exchanges were then scored as *twins* (an exchange at the same place in both sister chromosomes) or *singles*. Results are given for those chromosome pairs where it was considered sisters were unequivocaly chosen and for those where this was possibly ambiguous (Table 15).

Table 15.

Frequency of single and twin sister chromatid exchanges in M chromosome pairs of tetraploid cells

Treatment	Unequivocal pairs		Ambiguous pairs	
	Single exchanges	Twin exchanges	Single exchanges	Twin exchanges
A	101	48	69	32
B	59	34	30	17
C	18	9	10	9
Total	178	91	109	58
χ^2 2 d.f.	0.47	0.80>P>0.70	2.38	0.50>P>0.30
Number of chromosome pairs	103		76	

Correction for "false" twins

Since there is a relatively high frequency of sister chromatid exchanges per M chromosome it is probable that "false" twins will be scored. For a true sister chromosome pair these would result from two single exchanges occurring at the second division in similar regions of both chromosomes.

To obtain an estimate of this error, "false" twins and singles were scored in diploid second division cells. In such cells the two M chromosomes are non-sisters in origin i.e. no true twins are possible. An analysis of 77 pairs of M chromosomes in diploid cells gave *197* single exchanges and *30* "false" twin exchanges (a ratio of 6.57 : 1). Therefore of a total of *257* exchanges, *60* have been *mis-scored* as twin exchanges. This data can be used to obtain new estimates of single and twin exchanges.

If x and y are the frequency of "false" twin and single exchanges respectively, then the error due to "false" twins will be equal to $\frac{2x}{2x+y}$. Paired non-sister M chromosomes will give "false" twins from three origins

(i) Paired first division exchanges

(ii) A first division exchange in one chromosome paired with a second division exchange in the other.

(iii) Paired second division exchanges.

If p is the probability of occurrence of a sister chromatid exchange at the first division and p' the probability of an exchange at the second division, then the above events will correspond to p^2, $2\ p\ p'$ and $(p')^2$ respectively.

Marin and Prescott (1964) and Brewen and Peacock (in press) have shown that the probability of an exchange at the first division is equal to the probability of an exchange at the second division. Therefore $p = p'$ and the above events will occur in the ratio 0.25 : 0.50 : 0.25 respectively.

The error term $\frac{(2x)}{(2x+y)}$ will apply if all pairs of M chromosomes in tetraploid cells are chosen incorrectly. However if <u>all</u> pairs are chosen correctly then only $(p')^2$ events can contribute "false" twins. In this case the correction factor will be equal to $\frac{1}{4}\ \frac{(2x)}{(2x+y)}$ and will be applicable to the data from unequivocal pairs in Table 15.

If chromosome pairs are chosen at random then the correction factor will be $\frac{2}{3}\ \frac{(2x)}{(2x+y)} + \frac{1}{3}\ [\frac{1}{4}\ \frac{(2x)}{(2x+y)}]$ which is equal to $\frac{3}{4}\ \frac{(2x)}{(2x+y)}$, and will be applicable to the data from ambiguous pairs in Table 15.

The correction factor is applied to the data from tetraploid cells in the following fashion.

If n is the observed number of single exchanges then N, the adjusted number of single exchanges is equal to

$n\ (\frac{1}{1 - C.F.})$ where $C.F.$ is the appropriate correction factor. Thus if t were the observed number of twin exchanges, the adjusted number T would be equal to $t - (\frac{N - n}{2})$.

$$\text{Minimum correction factor} = \frac{1}{4}\ (\frac{2\ x}{2\ x + y})$$
$$= 0.06$$

$$\text{Maximum correction factor} = \frac{3}{4}\ (\frac{2\ x}{2\ x + y})$$
$$= 0.17$$

Actual and adjusted data are presented in Table 16.

All adjustments will be an overestimate of the true situation since as well as second division exchanges being paired as "false" twin exchanges they will also remove true twins. A second division exchange occurring at the same region as a first division exchange will remove a true twin and result in a single exchange. Therefore if the loss of true twins from superimposed second division exchanges is ignored the corrections used will be the maximum applicable. Even the application of this maximum correction to the total data does not result in a significant deviation from the 2 : 1 expectation.

Table 16.

Frequencies of single and twin sister chromatid exchanges in paired M chromosomes from tetraploid cells

		Single exchanges	Twin exchanges	Ratio	χ^2 1 d.f.	P
Unequivocal pairs	actual	178	91	1.96 : 1	0.04	0.90 - 0.80
	adjusted	189	85.5	2.21 : 1	0.55	0.50 - 0.30
Ambiguous pairs	actual	109	58	1.88 : 1	0.14	0.80 - 0.70
	adjusted	132	46.5	2.88 : 1	4.26	0.05 - 0.02
Total pairs	actual	287	149	1.93 : 1	0.17	0.70 - 0.50
	adjusted	321	132	2.43 : 1	3.50	0.10 - 0.05

f. Single and twin isolabelling segments

Isolabelling was scored as segments; being either twin or single segments on sister M chromosome pairs. The data obtained is given in Table 17 combined from unequivocal and ambiguous pairs.

Table 17.

Frequencies of single and twin isolabel segments in M chromosomes of tetraploid cells

Treatment	Single segments	Twin segments
A	88	38
B	88	36
C	41	8
Total	217	82

Between treatments $\chi^2_{2\ d.f.}$ 3.67 0.20>P>0.10

Data from 179 pairs of M chromosomes; giving a ratio of 2.65 : 1. An estimate of "false" twin isolabel segments was also made. Analysis of 30 pairs of non-sister M chromosome pairs from diploid cells gave 47 single and 9 "false" twin isolabel segments i.e. a ratio of 5.22 : 1. Since the method of scoring tended to overestimate isolabelling to an unknown extent, a correction factor was not applied to the data, however resultant ratios would deviate considerably from that found for sister chromatid exchanges.

g. Position of sister chromatid exchanges on S chromosomes

In order to determine if exchanges were localised to broad equal regions of the S chromosomes, exchanges were scored as being proximal, distal or median with regard to the centromere from 2 samples of S chromosomes.

Table 18.

Position of sister chromatid exchange in S chromosomes

	Proximal	Median	Distal	χ^2 2 d.f.	P
All chromosomes	85	106	99	2.36	0.50 - 0.30
Chromosomes with one exchange	95	144	125	10.17	<0.01

h. Centromere "exchanges" in M chromosomes

Apparent exchanges at the centromere can arise from twisting at the centromere. The centromere regions of M chromosomes are frequently seen stretched or possibly broken. A sample of M chromosomes without isolabelling where exchanges or apparent exchanges could be scored without this complication was therefore selected. From 56 M chromosomes with a mean frequency of non-centromere sister chromatid exchanges of 3.75 per chromosome, 19 had centromere "exchanges".

Therefore of a total of 229 exchanges, 8.3% were found at the centromere.

Although the centromere regions of many M chromosomes analysed for single and twin exchanges were unlabelled, of those that were adequately labelled 16 single and 9 twin "exchanges" were seen.

Discussion

Throughout this report the term "sister chromatid exchange" as used by Taylor (1958) has implied that the cross-over labelling patterns found on sister chromatids of second division chromosomes have resulted from an actual exchange of chromatid segments. This has been accepted by subsequent workers *e.g.* Peacock (1963) and Marin and Prescott (1964).

However Stahl (1964) has pointed out a possible alternative explanation. He has proposed that, following a chromatid break sub-units rejoin at random *within* a chromatid (*without* any exchange between the two sister chromatids). If sister chromatids break at the same level and such random rejoining of sub-units takes place the ratio of single to twin "exchanges" at the second post labelling metaphase will be 2 : 1 (*cf.* Taylor 1958, 2 : 1 for restricted rejoining). "This situation that derives without chromatid exchange and with assumed identity of

sub-units mimics chromatid exchange with non-identity of sub-units. Thus an independent demonstration of chromatid exchange is needed, but seems to be lacking at this time" (Stahl, 1964 p.80).

Peacock and Brewen (in manuscript) have provided this demonstration by scoring the distribution of labelled segments of third division endoreduplicated chromosomes in Chinese Hamster cells. This method enabled them to distinguish between the Stahl and Taylor hypothesis. They concluded that sister chromatid exchanges are the visible result of a physical exchange of sister chromatid segments.

Sister chromatid exchanges

a. *Frequency of exchanges*

As can be seen from Table 19, sister chromatid exchanges have been observed in a wide range of organisms, and it is obvious that the frequency of exchanges per chromosome varies considerably from organism to organism. Taylor (1958) observed that in *Bellevalia romana* the frequency of exchanges was nearly proportional to chromosome length. The present study also shows close agreement between exchange frequency and chromosome length.

Results from experiments with *Vicia faba* are variable. Taylor *et al* (1957) reported that they found "occasional chromatid exchanges", while Peacock (1963) and this study report different frequencies of exchange per chromosome,

Table 19.

Sister chromatid exchange frequencies in a number of organisms

Organism	Chromosome class	Number of chromosomes scored	Mean sister chromatid exchanges per chromosome	Ratio of chromosome lengths	Ratio of S.C.E.[a] per chromosome	Author
Bellevalia romana	I (large metacentric)	72	1.21	1.0	1.0	Taylor (1958)
	II (medium subtelocentric)	52	0.74	0.8	0.6	
	III, IV (small metacentrics)	80	0.84	0.7	0.7	
Allium cepa	Approximately equal long chromosomes	112	0.91			Zweidler (1964)
Chinese hamster cell line CHEF-125	I (largest metacentric)	335	0.74			Marin and Prescott (1964)
	overall	1061	0.28			
Potorous tridactylis (marsupial cell line)	large group	156	0.54			Walen (1965)
	small group	64	0.13			
Human leucocytes	Group A	238	0.41	1.0*	1.0	Herreros and Gianelli (1967)
	B	106	0.32	0.8	0.8	
	C	878	0.30	0.6	0.7	
	D	462	0.21	0.5	0.5	
	E	330	0.09	0.4	0.2	
	F	218	0.04	0.3	0.1	
	G	314	0.05	0.2	0.1	
Goniaea australasiae (Orthoptera; Acrididae)	I (large autosomes)	180	1.14	0.9	0.8	Peacock (1968)
	II (medium autosomes)	335	0.82	0.6	0.6	
meiotic chromsomes	X (largest chromosome)	32	1.45	1.0	1.0	
*Vicia faba***	S subtelocentric	776	0.73	1.0	1.0	Peacock (1963)
	M metacentric	144	1.29	2.4	1.8	
Vicia faba (*var* Coles Dwarf Prolific)**	S	814	1.64	1.0	1.0	(this study)
	M	120	4.05	2.4	2.5	

* estimated from Tjio and Puck (1958).

** data bulked over treatments and excluding all chromosomes with isolabelling.

however trends are consistent throughout (see Appendix II). The reason for these discrepancies is unknown. The constancy between experiments in this study (which were carried out in two different laboratories) is good.

b. *Effect of colchicine*

Taylor (1959) reported a significant effect of time of availability of colchicine on exchange frequency in *Bellevalia romana*. Peacock (1963) reported that colchicine did not effect exchanges in *Vicia faba*. These results show that there may be a slight colchicine effect, in that, when colchicine is provided before and during label sister chromatid exchanges are slightly increased (*contra* Taylor, 1959). Table 3 shows that for S chromosomes there is a significant difference between treatments; however the results overall (Tables 3, 4 and 5) indicate little effect (if any) in *Vicia* in contrast to that in *Bellevalia*.

(The results of Peacock (1963) with isolabelled chromosomes removed from the analysis are reported in Appendix II.)

c. *Exchange distribution*

If sister chromatid exchanges arise as independent events, the distribution of 0, 1, 2 ..... exchanges per chromosome should follow a Poissonian form. The total

distribution of exchanges for M chromosomes is considered in Table 7, while S chromosomes are considered for each treatment separately, since there is a significant difference between treatments. Two of the four distributions for the S chromosomes deviate significantly from a Poissonian form. The variance/mean ratios given in Appendix I for individual distributions indicate an under-dispersion of data.

The deviations from the Poisson could be interpreted as meaning that the occurrence of a sister chromatid exchange reduces the prospect of a further exchange. Alternatively a non-random distribution of exchanges along the length of the chromosomes could be responsible for the deviations from the Poisson form. However observations of the approximate positions of sister chromatid exchanges along the length of S chromosomes (Table 18) do not support the possibility of non-random exchange distributions. Where S chromosomes have one sister chromatid exchange there is a tendency for this not to occur proximal to the centromere.

Another explanation however, is that some multiple sister chromatid exchanges which were not adequately resolved by the autoradiographic technique have been interpreted as isolabelling. A small number of such mis-scorings could cause the observed deviations from the

Poisson distribution. If the explanation is accepted exchange events are independent.

Single to twin sister chromatid exchange ratios

Peacock and Brewen (in manuscript) have shown that sister chromatid exchanges involve the physical exchange of chromatid segments. However given this mode of origin a number of hypotheses giving expectations of the ratio of singles to twins can be formulated.

Taylor (1958) considered that sister chromatid exchanges were spontaneous sister strand cross-overs and derived his expectations accordingly. He realised (Taylor, 1959) that exchanges could be due to endogenous radiation from the incorporated tritium, however he rejected this possibility in the case of *Bellevalia* since he found that exchanges were influenced by colchicine; the higher frequency of exchange in the first interphase not persisting in all experiments. Wolff (1964) pointed out that the ratio originally found by Taylor (1958) i.e. 0.37 : 1, was compatible with that to be expected if exchanges were radiation induced and followed dose square kinetics. He contended that if colchicine could be invoked to influence exchanges (presuming spontaneity) then there is no *a priori* reason why colchicine could not be invoked to influence radiation induced exchanges.

Brewen and Peacock (in press) have provided some support for Wolff's contention that exchanges are radiation induced, since they found that tritiated thymidine induces sister chromatid exchange in a human ring chromosome.

Radiation induced exchanges could follow either linear (one hit) or dose square (two hit) kinetics. A summary of single : twin expectations for these various possibilities is given in Table 20.

Table 20.

Expectations of single to twin sister chromatid exchange ratios

	Exchanges occurring independent of H^3 thymidine dose	Exchanges induced by tritium radiation; following dose square kinetics	Exchanges induced by tritium radiation; following linear kinetics
Sub-units rejoining non-randomly (= polarity)	2 : 1	1 : 2	1 : 1
Sub-units rejoining at random (= non-polarity)	10 : 1	4 : 1	6 : 1

The ratios that Taylor (1959) obtained varied with colchicine treatment from approximately 1 : 2, through 1 : 1, to 2 : 1. Since these ratios constituted a significant departure from a 10 : 1 ratio Taylor (1959 p.70) concluded: "The two strands of a chromosome or a chromatid are structurally different in a way that prevents re-union of unlike strands". In contrast to this study, in his original experiments Taylor (1958) found no twin exchanges (i.e. "false" twins) in diploid second division cells. Hence any correction that could be applied to the data would result in little change, and demonstrates the confidence with which sister chromosome pairs were chosen.

Therefore Taylor provided a further parallel between the behaviour of chromatid sub-units and the DNA double helix; from the demonstration of semi-conservative segregation of DNA at the chromosomal level (Taylor *et al* 1957).

Obviously this point is of considerable importance in drawing inferences about chromosome structure. The relative paucity of Taylor's (1959) data and the complexing effect of colchicine tend to negate to some extent confidence in the interpretation of Taylor's results.

Other data bearing on single : twin exchange ratios is that of Walen (1965) and Herreros and Gianelli (1967). A note giving ratios but no data has been presented by Sparvoli, Gay and Kaufmann (1966).

Walen (1965) examined sister chromatid exchanges in endoreduplicated cells of *Potorous tridactylis*. In such cells homologous chromosomes occur in pairs so there is no ambiguity in selecting sister pairs. She found *63* single exchanges and *15* twin exchanges on 55 chromosome pairs, i.e. 4.2 : 1. In a partial breakdown of the results obtained, Walen (1965, Fig. 10, p.923) records exchanges occurring at the centromere or elsewhere along the length of the chromosome from 41 of the original 55 chromosome pairs. From this table it is obvious that exchanges at the centromere (particularly single exchanges) occur in a disproportionately higher frequency (>20%) than would be expected if exchanges were randomly distributed along the chromosomes. Twisting at the centromere can mimic an exchange, however Walen (1965 p.926) states: "the lack of flexures at the centromere in this material, implying a relatively rigid continuity, implies that erroneous classification from this source has probably contributed little to the observed results."

Herreros and Gianelli (1967) have analysed a large number of human endo-reduplicated chromosomes for single

and twin exchanges. Table 21 presents their data separately for centromere and non-centromere exchanges.

Table 21.

Data from Herreros and Gianelli (1967)

	Single exchanges	Twin exchanges	Ratio
Centromere exchanges	191	55	3.5 : 1
Non-centromere exchanges	288	128	2.2 : 1
Total	479	183	2.6 : 1

$\chi^2_{1\ d.f.}$ 5.46 $0.02>P>0.01$

This contingency analysis shows that a significantly different ratio is obtained for exchanges at the centromere, precluding bulking the data to obtain a definitive final ratio. An examination of exchange events shows that the mean frequency (over chromosome groups) of exchanges at the centromere is 38%, which implies a non-random distribution of exchanges along the chromosome (and varies from 26 to 62% between groups) with exchanges localised at the centromere. However as stated previously twisting at the centromere can mimic an exchange therefore actual exchanges at the centromere cannot be ascertained. The unknown reason for the high frequency of centromere exchanges in both the results of Walen (1965) and Herreros

and Gianelli (1967) negate to some extent the influence of these data in a consideration of the organisation of chromosomal sub-units.

Marin and Prescott (1964) examined third division metaphases in a Chinese hamster cell line and found that, "one fifth of all second cycle exchanges scored in third division metaphases took place at the centromere" (p.166) in chromosome I (the largest metacentric). Centromere exchanges were not scored in second division exchanges since they could have arisen by twists at the centromere. This is unfortunate since it would have provided an estimate of centromere twisting in these comparatively large chromosomes. Cuevas-Sosa (1967) examined colchicine anaphase chromosomes of human lymphocytes and a neoplastic cell line for exchanges at the centromere. Therefore chromatids were separated at the centromere reducing considerably the prospect of an influence of centromere twisting. Although reporting sister chromatid exchanges, their frequencies are not recorded, therefore there is no sound basis for comparison with his finding of 48 centromere exchanges in 2300 lymphocyte chromosomes (50 cells) and 49 centromere exchanges in 3750 neoplastic cell line chromosomes (estimated because chromosome numbers differed from cell to cell.) Cuevas-Sosa's (1967) conclusion that sister chromatid exchanges "could happen"

(p.339) at the centromere does not however indicate that the frequency of exchanges at the centromere was considerable in relation to those along the remainder of the chromosomes.

Sparvoli, Gay and Kaufmann (1966) analysed third division prophase chromosomes of *Haplopappus gracilis* and found a ratio of single to twin exchanges of 10 : 1 (no data given therefore possible influences of colchicine and/or centromere exchanges cannot be assessed).

The frequency of "false" twin exchanges will be influenced by exchange frequency per chromosome which is itself proportional to chromosome length. However the removal of true twins by superimposed second division exchanges could possibly negate this effect of "false" twins to an unknown extent.

Experiments to date have produced single to twin sister chromatid exchange ratios ranging from 1 single to 2 twins; to 10 singles to 1 twin, providing no clear support for any one ratio given in Table 20.

Single and twin sister chromatid exchanges in *Vicia faba*

The contingency analyses show that colchicine has no influence on the frequency of single and twin exchanges and also there is no difference between the two sets of data ($\chi^2_{1\ d.f.}$ 0.05, 0.90>P>0.80). Applying the maximum

correction factor to the data from Table 15 gives a final ratio of 321 single exchanges to 132 twin exchanges (Table 16) i.e. a ratio of 2.43 : 1, which is not significantly different from a 2 : 1 ratio ($\chi^2_{1\ d.f.}$ 3·50, 0.10>P>0.05). If a higher frequency, than expected from random selection, of "ambiguous" chromosome pairs were true sister pairs, as seems likely by the closeness of the ratio for unequivocal and ambiguous chromosome pairs, then the final adjusted ratio would be closer to 2 : 1 than that obtained. This is reinforced by the fact that the ratio obtained from ambiguous pairs deviated from the "false" twin ratio (6.57 : 1) more than would be expected if selection had been at random. Therefore in *Vicia faba* sister chromatid exchanges occur independently of tritium dose and sub-units are dissimilar and exhibit polarity. This conclusion is compatible with part of the data of Taylor (1959) and part of that of Herreros and Gianelli (1967). Taylor's results were complicated by the effect of colchicine, which does not influence *Vicia* data, while that of Herreros and Gianelli (1967) was complicated by the different ratios obtained for centromere single and twin exchanges. In *Vicia* results indicate that the centromere is not a region with a preponderance of exchanges, (in M chromosome); while although data is sparse (16 singles to 9 twins) there is no evidence for a

different single : twin ratio at the centromere from that obtained. Frequently the M chromosomes used for scoring single and twin exchanges were not labelled at the centromere, indicating it is one of the later regions to be replicated (Evans, 1964).

The data obtained is therefore compatible with the conclusion that the two chromosome replication sub-units are not identical, in an analagous manner to the two chains of the DNA molecule.

Isolabelling

La Cour and Pelc (1958) were the first to report isolabelling at the second division in *Vicia faba*. Peacock (1963) carried out an extensive investigation of labelling on *Vicia* chromosomes and reported up to 33% and 22% isolabelling of M and S chromosomes respectively. Iordanskii (1964) also reported isolabelling in *Vicia*. Taylor (1958) found a few cases of isolabelling in *Bellevalia romana*; as did Walen (1965) in *Potorous tridactylis*, Gay (1965) in *Vicia, Crepis, Allium* and *Haplopappus* and Cuevas Sosa (1967) in human leucocytes. Gay (1966) found 20% and 5% isolabelling of the M and S chromosomes respectively in *Haplopappus gracilis*, while twin isolabelled segments were also reported.

This study reports a high frequency of isolabelling, showing an increased incidence with increased chromosome length and/or sister chromatid exchange frequency. Neither the frequency of isolabelling nor the type of isolabelling were influenced by time of application of colchicine (Tables 1, 2, 13 and 14), in agreement with Peacock (1963).

Peacock (1963) considers four possible origins of isolabelling.

(i) Residual isotope incorporation

Evens (1964) has shown that in *Vicia* root tips a supply of H^3 thymidine is not found a few minutes after removal of the isotope. He showed that the use of a cold thymidine chase extended the period of availability of isotope. A chase was used by Peacock (1963) but not in the present experiments.

(ii) Interchromosomal exchange

Taylor (1962) suggested that exchanges between the four chromatids of adjacent homologous chromosomes might result in isolabelling. Wolff (1964) also put forward this possibility. Such exchanges would result in "iso-unlabelled" segments on one homologue. Since twin isolabelling has been observed in reasonably high frequencies this explanation is untenable; also intercalary isolabelling has been

observed in high frequencies (*contra* Wolff's 1964 expectation).

(iii) Multiple exchanges

The location of grains in relation to the point of origin of the β ray from H^3 decay is at times imprecise, in that grains may not lie directly over the region of origin (Perry 1965). Hence grains may appear opposite each other on different chromatids following an exchange event. Multiple exchanges in a region will enhance this grain scatter, so isolabelling could therefore be scored. In order to obtain a uniform basis of scoring, chromosome labelling patterns were drawn and scored as they actually appeared. No attempt was made to see if grain overlap was a consequence of a sister chromatid exchange event. However three grains had to be present before labelling was accepted on any chromatid segment. Therefore it is most likely that some, particularly multiple exchanges, have been incorrectly classified as isolabelling. While this explains the deviation from the Poisson expectation of exchange distribution, it is felt that this mis-scoring contributed to only a fraction of total isolabelling.

(iv) Polyneme structure

If the chromosome were composed of more than one lateral strand of DNA, both segregational and isolabelling could be obtained at the second division, i.e. there is a lateral multiplicity of DNA per chromatid.

Peacock (1963, 1965) puts forward models demonstrating segregation of multi-stranded chromosomes and explains isolabelling on the basis of lateral multiplicity of *Vicia* chromosomes.

Taylor (1966) has presented a model of chromosome duplication which is adaptable to either a single or a multi-stranded chromatid.

Although the incidence of isolabelling increases with chromosome length (as do sister chromatid exchanges) the scoring of isolabelling in terms of exchange events introduces considerable heterogeneity in the replicate experiments of treatment A (see Appendix I), and also results in considerable departure of exchange distributions from the Poisson form (Tables 11 and 12).

The finding of twin and single isolabel segments indicates that they arose from first and second division events respectively, in an analagous manner to sister chromatid exchanges. The analysis of twin and single isolabel segments however shows considerable variation.

The relatively high frequency of "false" twin isolabel segments and the uncertainty with regard to actual isolabel segments make the computation of decisive ratios meaningless.

Also it may be incorrect to equate isolabel events with exchange events. Therefore apart from stating that a high incidence of isolabelling has been found on second division chromosomes, that incidence increases with chromosome length and/or exchange frequency, and that isolabelling can occur in twin and single segments, this study is unable to provide any information which could confirm or deny Peacock's (1963) explanation of isolabelling on the basis of chromosomal DNA lateral multiplicity.

REFERENCES

Anderson, R.L. and T.A. Bancroft, 1952. Statistical Theory in Research. McGraw-Hill: New York.

Brewen, J.G. and W.J. Peacock, 1958. The effect of tritiated thymidine on sister chromatic exchange in a ring chromosome. (In press).

Busch, H., W.C. Starbuck, E.J. Singh and Jae Suk Lo, 1964. Chromosomal proteins. In, The role of chromosomes in development, Ed. M. Locke : 51-71. Academic Press, New York.

Cuevas-Sosa, A., 1967. Crossing-over and the centromere. Cytogenetics *6*: 331-341.

Darlington, C.D., 1947. Nucleic acid and the chromosomes. Symp. Soc. Exp. Biol. *1*: 254-269.

1960. Chromosomes and the theory of heredity. Nature. *187* : 892-895.

Evans, J.H., 1964. Uptake of H^3 thymidine and patterns of DNA replication in nuclei and chromosomes of *Vicia faba*. Exptl. Cell Res. *35* : 381-393.

Ficq, A., 1959. Autoradiography. In, The Cell. Eds. J. Brachet and A.E. Mirsky *1* : 67-90. Academic Press, New York.

Filner, P., 1965. Semi-conservative replication of DNA in a higher plant cell. Exptl. Cell Res. *39* : 33-39.

Gay, H., 1965. Organisation of chromosomes in higher forms. Carnegie Inst. Wash. Year Book *64* : 539-554.

1966. Chromosome organisation in Eucaryotes. Carnegie Inst. Wash. Year Book *65* : 581-584.

Haut, W.F. and J.H. Taylor, 1967. Studies of bromouracil deoxyriboside substitution in DNA of bean roots (*Vicia faba*). J. Mol. Biol. *26* : 389-401.

Herreros, B. and F. Gianelli, 1967. Spatial distribution of old and new chromatid sub-units and frequency of chromatid exchanges in induced human lymphocyte endoreduplications. Nature, *216* : 286-288.

Huberman, J.A. and G. Attardi, 1966. Isolation of metaphase chromosomes from He La cells. J. Cell. Biol. *31* : 95-105.

Iordanskii, A.B., 1964. An autoradiographic study of reproduction of chromosomes in *Vicia faba*. Doklady Akad. Nauk S.S.S.R. *158* : 192-195.

Jackson, W.D. and H.N. Barber, 1958. Patterns of chromosome breakage after irradiation and ageing. Heredity, *12* : 1-25.

Kimball, J.W., 1965. Biology. Addison-Wesley, Reading, Massachusetts.

La Cour, L.F. and S.R. Pelc, 1958. Effect of colchicine on the utilisation of labelled thymidine during chromosomal reproduction. Nature, *182* : 506-508.

1959. Effect of colchicine on the utilisation of thymidine labelled with tritium during chromosomal reproduction. Nature, *183* : 1455-1456.

Lewis, K.R. and B. John, 1963. Chromosome marker. J. & A. Churchill, London.

Maio, J.J. and C.L. Schildkraut, 1967. Isolated mammalian metaphase chromosomes. I. General characteristics of nucleic acids and proteins. J. Mol. Biol. *24* : 29-39.

Marin, G. and D.M. Prescott, 1964. The frequency of sister chromatid exchanges following exposure to varying doses of H^3 thymidine or X-rays. J. Cell Biol. *21* : 159-167.

Martin, P.G. and R. Shanks, 1966. Does *Vicia faba* have multi-stranded chromosomes? Nature, *211* : 650-651.

Mather, K., 1965. Statistical analysis in biology. Methuen, London.

Meselson, M. and F.W. Stahl, 1958. The replication of DNA in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S. *44* : 671-682.

Mirsky, A.E., 1947. Chemical properties of isolated chromosomes. C.S.H. Symp. Quant. Biol. *12* : 143-146.

Peacock, W.J., 1963. Chromosome duplication and structure as determined by autoradiography. Proc. Natl. Acad. Sci. U.S. *49* : 793-801.

1965. Chromosome replication. Natl. Cancer. Inst. Monograph *18* : 101-129.

1968. Replication, recombination and chiasmata in *Goniaea australasiae* (Orthoptera : Acrididae). Genetics (in press).

Peacock, W.J. and J.G. Brewen, 1968. Chromosome sub-units and sister chromatid exchange. (In manuscript).

Pearson, E.S. and H.O. Hartley, 1954. Biometrika tables for statisticians. I. Cambridge University Press, Cambridge.

Perry, R.P., 1965. Quantitative autoradiography. In, Methods in Cell Physiology. Ed. D.M. Prescott : 305-326. Academic Press, New York.

Prescott, D.M., 1961. The growth-duplication cycle of the cell. Int. Rev. Cytology. *11* : 255-282.

Reichard, P. and B. Estborn, 1951. Utilisation of desoxyribosides in the synthesis of polynucleotides. J. Biol. Chem. *188* : 839-846.

Sager, R. and F.J. Ryan, 1961. Cell heredity. J. Wiley & Sons, New York and London.

Simon, E.H., 1961. Transfer of DNA from parent to progeny in a tissue culture line of human carcinoma of the cervix (strain He La). J. Mol. Biol. *3* : 101-109.

Sparvoli, E., H. Gay and B.P. Kaufmann, 1966. Distribution of incorporated H^3 thymidine in mitotic chromosomes of *Haplopappus gracilis*. 3rd Int. Cong. Rad. Res. Cortina abst. 828:208.

Stahl, F.W., 1964. The mechanics of inheritance. Prentice-Hall, New Jersey.

Stedman, E., and E. Stedman, 1947. The function of deoxyribose nucleic acid in the cell nucleus. Symp. Soc. Exp. Biol. *1* : 232-251.

Taylor, J.H. 1965. Autoradiography at the cellular level. In, Physical techniques in biological research. Eds. G. Oster and E.W. Pollister, *3* : 546-577.

1958. Sister chromatid exchanges in tritium-labeled chromosomes. Genetics, *43* : 515-529.

1959. The organisation and duplication of genetic material. Proc. Xth Int. Cong. Gen. *1* : 63-78.

1962. Chromosome reproduction. Int. Rev. Cytology, *13* : 39-73.

1963. The replication and organisation of DNA in chromosomes. In, Molecular genetics Part I, ed. J.H. Taylor : 65-111. Academic Press, New York.

1966. The duplication of chromosomes. In, 3 Wissenschaftliche Konferenz der Gesellschaft Deutscher Naturforscher und Arzte. Ed. P. Sitte : 9-26. Springer-Verlag, Heidelberg.

Taylor, J.H., P.S. Woods and W.L. Hughes, 1957. The organisation and duplication of chromosomes as revealed by autoradiographic studies using tritium labelled thymidine. Proc. Natl. Acad. Sci. U.S. *43* : 122-128.

Tjio, J.H. and T.T. Puck, 1958. The somatic chromosomes of man. Proc. Natl. Acad. Sci. U.S. *44* : 1229-1237.

Walen, K.H., 1965. Spatial relationships in the replication of chromosomal DNA. Genetics, *51* : 915-929.

Watson, J.D., 1965. Molecular biology of the gene. W.A. Benjamin, New York and Amsterdam.

Watson, J.D. and F.H.C. Crick, 1953a. A structure for desoxyribose nucleic acids. Nature, *171* : 737-738.

Watson, J.D. and F.H.C. Crick, 1953b. Genetical implications of the structure of desoxyribonucleic acid. Nature, *171* : 964-966.

Watson, J.D. and F.H.C. Crick, 1953c. The structure of DNA. C.S.H. Symp. Quant. Biol. *18* : 123-131.

Wilkins, M.H.F., A.R. Stokes and H.R. Wilson, 1953. Molecular structure of desoxypentose nucleic acids. Nature, *171* : 738-740.

Wilson, E.B., 1925. The cell in development and heredity, 3rd edition. MacMillan, New York.

Wolff, S., 1964. Are sister chromatid exchanges sister strand crossovers or radiation induced exchanges? Mutation Res. *1* : 337-343.

Woods, P.S. and M.A. Schairer, 1959. Distribution of newly synthesised deoxyribonucleic acid in dividing chromosomes. Nature, *183* : 303-305.

Zweidler, A., 1964. Die replikation der chromosomen von *Allium cepa*. Arch. Klaus-Stift. Vererb Forsch. *39* : 54-64.

Appendix I

Results from replicate experiments for treatments A and B

Table 1.

Label distribution in chromosomes

Treatment	Chromosome type	Replicate experiment	Chromosomes with label segregation	Chromosomes with iso-labelling	Total chromosomes scored	% iso-labelling
A	S	I	105	71	176	40.3
		II	97	71	168	42.3
		III	122	108	230	47.0
		Total	324	250	574	43.6
		χ^2 2 d.f.	1.94	0.50 > P > 0.30		
	M	I	15	35	50	70.0
		II	19	37	56	66.1
		III	22	54	76	71.0
		Total	56	126	182	69.2
		χ^2 2 d.f.	0.28	0.90 > P > 0.80		
B	S	I	141	92	233	39.5
		II	87	97	184	52.7
		Total	228	189	417	45.3
		χ^2 1 d.f.	7.26	0.01 > P > 0.001		
	M	I	11	27	38	71.0
		II	15	58	73	79.5
		Total	26	85	111	76.6
		χ^2 1 d.f.	1.02	0.50 > P > 0.30		

Table 2

S. chromosomes. Distribution in sister chromatid exchange categories.

	Number of exchanges per chromosome						Number of chromosomes	Mean no. of exchanges/chromosome (S.E. in brackets)	$\frac{Vx}{\bar{x}}$	$\frac{\Sigma(x-\bar{x})^2}{\bar{x}}$	C	P	χ^2	d.f.	P
	0	1	2	3	4	5									
Treatment A															
I actual	19	34	37	11	3	1	105	1.50 (0.10)	0.75	77.50	1.94	0.10–0.05			
Poisson	23.4	35.1	26.4	13.2	4.9	1.9							6.64	3	0.10–0.05
II actual	20	34	31	11	1	-	97	1.37 (0.10)	0.69	66.16	2.39	x			
Poisson	24.6	33.8	23.2	10.6	3.6	1.2							4.23	2	0.20–0.10
III actual	16	43	39	17	5	2	122	1.66 (0.10)	0.73	88.89	2.26	x			
Poisson	23.1	38.5	32.0	17.7	7.3	3.3							4.81	3	0.20–0.10
	Actual data between replicates $\chi^2_{6d.f.}$ 4.23 0.70 > P > 0.50														
Treatment B															
I actual	19	41	45	22	10	4	141	1.82 (0.10)	0.83	115.70	1.55	0.20–0.10			
Poisson	23.3	42.0	37.8	22.7	10.4	4.9							2.31	3	0.70–0.50
II actual	13	22	27	21	3	1	87	1.80 (0.13)	0.77	66.49	1.62	0.20–0.10			
Poisson	14.4	25.9	23.3	14.0	6.4	3.0							7.91	3	x
	Actual data between replicates $\chi^2_{4d.f.}$ 4.44 0.50 > P > 0.30														
Treatment C							71		0.59	41.48	2.78	xx			
Treatment D							191		0.68	129.85	3.40	xxx			

x significant at 0.05 level xx significant at 0.01 level xxx significant at 0.001 level

Table 3

M. chromosomes. Distribution in sister chromatid exchange categories

	Number of exchanges per chromosome									Number of chromosomes	Mean No. of exchanges/chromosome (S.E.)	$\frac{Vx}{\bar{x}}$	$\Sigma\frac{(x-\bar{x})^2}{\bar{x}}$	P	χ^2	d.f.	P
	0	1	2	3	4	5	6	7	8								
Treatment A																	
I actual	1	0	2	5	2	3	2	0	0	15	3.60 (0.42)	0.75	10.44	0.30–0.20			
II actual	0	0	4	3	5	4	2	1	0	19	4.00 (0.34)	0.56	10.00	0.10–0.05			
III actual	0	1	0	5	4	8	1	2	1	22	4.54 (0.34)	0.56	11.77	x			
Sum actual	1	1	6	13	11	15	5	3	1	56	4.11 (0.21)						
Poisson	0.9	3.8	7.8	10.6	10.9	9.0	6.2	3.6	3.3						7.61	4	0.20–0.10
Treatment B																	
I actual	0	1	1	3	2	1	2	0	1	11	4.09 (0.61)	1.00	10.00	0.50–0.30			
II actual	0	1	3	3	3	2	3	0	0	15	3.75 (0.42)	0.71	9.90	0.30–0.20			
Treatment C										16		0.46	68.31	xxx			
Treatment D										22		1.22	25.53	0.80–0.70			

In all cases except that of the summation of Treatment A there were insufficient numbers for statistical test.

Table 4

S. chromosomes. Distribution in total exchange categories.

	Number of exchanges per chromosome						Number of chromosomes	Mean no. of exchanges/chromosome (S.E.)	$\frac{Vx}{\bar{x}}$	$\frac{\Sigma(x-\bar{x})^2}{\bar{x}}$	C	P	χ^2	d.f.	P
	0	1	2	3	4	5									
Treatment A															
I actual	19	75	57	17	6	2	176	1.5 (0.08)	0.64	112.46	3.7	xxx			
Poisson	36.9	57.7	45.1	23.4	9.1	3.8							20.62	3	xxx
II actual	20	67	47	26	7	1	168	1.62 (0.08)	0.69	114.58	3.16	xx			
Poisson	33.2	53.9	43.6	23.6	9.5	4.2							11.31	3	x
III actual	16	81	71	38	14	10	230	1.93 (0.08)	0.74	169.81	2.99	xx			
Poisson	33.4	64.4	62.2	40.0	20.0	10.0							16.48	4	xx
	Actual data between replicates $\chi^2_{8d.f.}$ 15.09 $0.10 > P > 0.05$														
Treatment B															
I actual	19	81	74	42	13	4	233	1.83 (0.07)	0.66	153.26	4.05	xxx			
Poisson	37.4	68.4	62.6	38.2	17.5	9.0							17.77	4	xx
II actual	13	62	56	44	8	1	184	1.87 (0.08)	0.59	108.49	4.43	xxx			
Poisson	28.3	53.0	49.7	30.9	14.4	7.6							25.47	4	xxx
	Actual data between replicates $\chi^2_{4d.f.}$ 3.35 $0.70 > P > 0.50$														
Treatment C															
actual	7	40	45	24	9	0	125	1.90 (0.09)	0.54	66.76	4.22	xxx			
Poisson	18.7	35.5	33.7	21.4	10.2	5.5							17.64	4	xx
Treatment D															
actual	21	131	108	49	14	3	326	1.74 (0.06)	0.59	190.41	6.00	xxx			
Poisson	57.2	99.6	86.7	55.2	16.9	10.5							44.59	4	xxx

Table 5

M. chromosomes. Distribution in total exchange categories.

	Number of exchanges per chromosome										Number of chromosomes	Mean no. of exchanges/chromosome	$\frac{Vx}{\bar{x}}$	$\frac{\Sigma(x-\bar{x})^2}{\bar{x}}$	C	P	χ^2	d.f.	P
	0	1	2	3	4	5	6	7	8	9									
Treatment A																			
I actual	1	4	4	15	9	8	9	0	0	0	50	3.74 (0.22)	0.67	33.05	1.87	0.10-0.05			
Poisson	1.2	4.4	8.3	10.4	9.7	7.2	4.5	2.4	1.1	0.7							4.48	4	0.50-0.30
II actual	0	1	7	12	13	10	7	4	2	0	56	4.27 (0.22)	0.64	35.36	2.13	x			
Poisson	0.8	3.3	7.1	10.2	10.9	9.3	6.6	4.0	2.1	1.7							2.13	4	0.80-0.70
III actual	0	3	2	8	12	20	10	15	3	3	76	5.20 (0.21)	0.64	48.47	2.44	x			
Poisson	0.4	2.2	5.7	9.8	12.8	13.3	11.5	8.6	5.6	6.3							12.96	6	x
	Actual data between replicates $\chi^2_{10d.f.}$ 29.63 xxx																		
Treatment B																			
I actual	0	1	2	5	12	8	5	4	1	0	38	4.58 (0.25)	0.53	19.49	2.42	x			
Poisson	0.4	1.8	4.0	6.2	7.1	6.6	5.0	3.3	1.9	1.7							6.09	4	0.20-0.10
II actual	0	3	4	15	14	17	12	4	2	1	73	4.60 (0.21)	0.69	50.02	2.04	x			
Poisson	0.7	3.4	7.8	11.9	13.7	12.6	9.7	6.3	3.6	3.3							6.97	5	0.30-0.20
	Actual data between replicates $\chi^2_{3d.f.}$ 2.31 0.70 > P > 0.50																		
Treatment C																			
actual	0	0	2	20	11	11	9	4	1	3	61	4.59 (0.22)	0.67	39.99	2.06	x			
Poisson	0.7	3.0	6.9	10.3	11.6	10.4	7.8	5.0	2.8	2.5							16.71	4	xxx
Treatment D																			
actual	0	3	6	19	14	9	7	5	1	1	66	4.20 (0.23)	0.83	53.91	1.08	0.30-0.20			
Poisson	1.0	4.2	8.7	12.2	12.8	10.8	7.5	4.5	2.4	1.8							7.26	5	0.30-0.20

Table 6.

Frequency of classes of isolabelling on isolabelled chromosomes

Treatment	Chromosome type	Replicate experiments	Class of isolabelling		
			Complete	Terminal	Intercalary
A	S	I	11	57	7
		II	8	52	18
		III	15	75	26
		Total	34	184	51
		χ^2 4 d.f.	6.60	0.20 > P > 0.10	
	M	I	3	17	24
		II	0	27	26
		III	1	43	41
		Total	4	87	91
		χ^2 2 d.f.	0.35	0.90 > P > 0.80	
B	S	I	6	72	19
		II	8	68	26
		Total	14	140	45
		χ^2 2 d.f.	1.40	0.50 > P > 0.30	
	M	I	0	24	17
		II	2	33	45
		Total	2	57	62
		χ^2 1 d.f.	2.36	0.20 > P > 0.10	

N.B. For M chromosome analysis completes were bulked with terminals.

Table 7.

Frequency of single and twin sister chromatid exchanges in pairs of M chromosomes from tetraploid cells

Treatment	Replicate experiment	Unequivocal pairs		Ambiguous pairs	
		Single exchanges	Twin exchanges	Single exchanges	Twin exchanges
A	I	40	11	29	9
	II	40	20	4	3
	III	21	17	36	20
	Total	101	48	69	32
	χ^2 2 d.f.	5.41	$0.10 > P > 0.05$	not applicable	
B	I	37	23	14	5
	II	22	11	16	12
	Total	59	34	30	17
	χ^2 1 d.f.	0.23	$0.70 > P > 0.50$	1.34	$0.30 > P > 0.20$

Table 8.

Frequency of single and twin isolabelled segments in M chromosome pairs of tetraploid cells

Treatment	Replicate experiment	Single segments	Twin segments
A	I	19	9
	II	26	7
	III	43	22
	Total	88	38
	χ^2 2 d.f. 1.73	0.50>P>0.30	
B	I	30	13
	II	58	23
	Total	88	36
	χ^2 1 d.f. 0.05	0.90>P>0.80	

Appendix II

Data extracted from the work of Peacock (1963); considering only those chromosomes without isolabel.

Treatment	S chromosomes	Sister chromatid exchanges per chromosome					Number of chromosomes	Mean no. of exchanges/ chromosome (S.E.)	$\frac{Vx}{\bar{x}}$	$\Sigma\frac{(x-\bar{x})^2}{\bar{x}}$	C	P	χ^2	d.f.	P
		0	1	2	3	4									
colchicine after label (A)	actual	118	87	50	3	0	258	0.76 (0.05)	0.84	217.24	1.85	1.0-0.9			
	Poisson	120.3	91.7	34.8	8.8	2.0							12.56	2	xx
colchichine before label (C)	actual	129	95	37	5	0	266	0.69 (0.04)	0.88	232.93	1.46	0.9-0.8			
	Poisson	133.4	92.1	31.8	7.3	1.5							2.73	2	0.30-0.20
colchicine 17 hrs after label (D)	actual	116	92	35	8	1	252	0.75 (0.05)	0.94	235.66	0.72	0.7-0.5			
	Poisson	119.0	89.2	33.4	8.4	1.7							0.36	2	0.90-0.80
	Actual data between treatments $\chi^2_{6\ d.f.}$ 5.51 0.50>P>0.30														
	M chromosomes														
colchicine after label (A)	actual	13	12	16	6	3	50	1.48 (0.16)	0.94	46.27	0.33	0.7-0.5			
	Poisson	11.5	16.8	12.5	6.1	2.6							2.56	2	0.30-0.20
colchicine before label (C)	actual	19	10	18	6	2	55	1.71 (0.16)	0.80	43.12	1.15	0.8-0.7			
	Poisson	9.9	17.1	14.6	8.3	4.1							13.67	2	xx
colchicine 17 hrs after label (D)	actual	14	10	14	4	0	42	1.19 (0.16)	0.87	35.69	0.66	0.7-0.5			
	Poisson	11.6	15.2	9.1	3.6	1.2							5.05	2	0.10-0.05
	Actual data between treatments $\chi^2_{6\ d.f.}$ 2.70 0.90>P>0.80														

Section II

Mitotic cycle determinations

Introduction

The rationale for the determination of the time that cells take to go from the mid-point of one mitosis to the mid-point of the next mitosis was provided by Howard and Pelc (1953). They divided the inter-mitotic period of cells into 3 phases; *viz.* G1 - presynthetic phase; S - DNA synthesis phase; G2 - postsynthetic phase (G = gap). The readily observable mitotic phase (M) is divided into the classical stages: prophase, metaphase, anaphase and telophase.

The labelled mitosis wave method (Quastler and Sherman 1951, Wimber 1960) was employed to obtain the duration of the mitotic cycle phases (G1, S, G2 and M). These may be deduced by studying the relative changes in the proportions of mitotic cells which are labelled at various time intervals (over at least one mitotic cycle) after H^3 thymidine treatment. Lima de Faria (1959) lists many of the earlier publications on studies of cell population kinetics using H^3 thymidine.

Experimental methods

I. *Vicia faba*

In order to obtain some familiarisation with the "per cent labelled mitoses" technique, a preliminary experiment was carried out in variable temperature conditions (19.5 ± 1.5°C); and after evaluation, a further experiment under controlled conditions (22 ± 0.1°C) was performed. Secondary roots of *Vicia faba* (var. Cole's Dwarf Prolific) were cultured and handled as before.

	Experiment A	Experiment B
Temperature	19.5 ± 1.5°C	22 ± 0.1°C
H^3 thymidine	2 µc./ml. for 1 hr. (S.A. 2.0 Cu/mM)	2 µc./ml. for 30 mins. (S.A. 3.0 Cu/mM)
root tips collected	4 per hour for 12 hours and then 2 hourly to 24 hours post label	3 per hour for 27 hours post label
Cells scored	Labelled : unlabelled metaphases; 100 cells per slide (where available) Results shown in Figure 1. where the mean and confidence limits at the 95% level are recorded for each fixation time.	Estimate of mitotic index. Labelled : unlabelled prophases, metaphases and ana-telophases. 300 mitotic cells per slide The % labelled prophase curve is presented in Figure 2. Individual root tip values at each time interval being given.

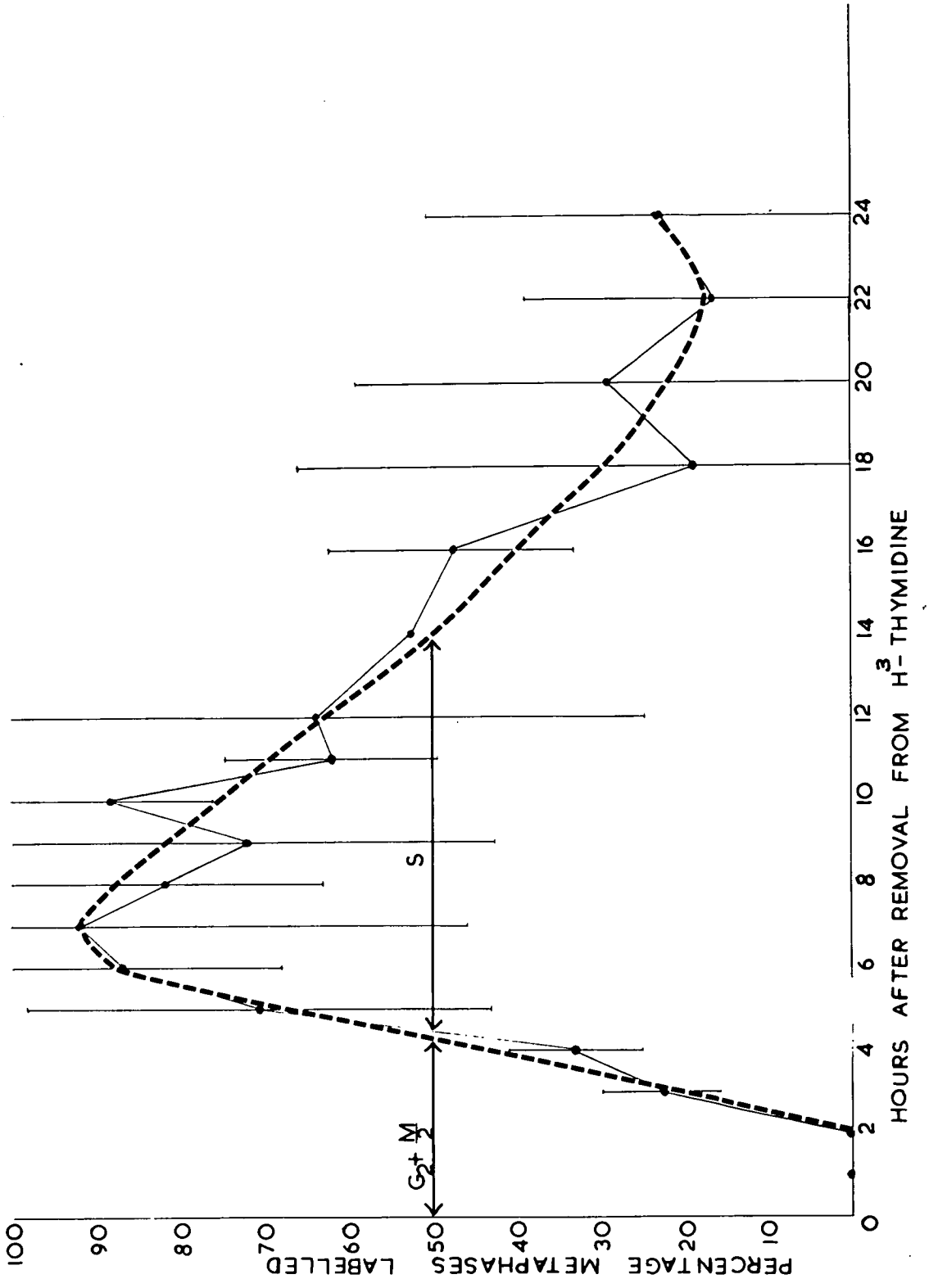


Figure 1. *Vicia faba* - The per cent labelled metaphase figures at various times after a 1 hour labelling period with H^3 thymidine.

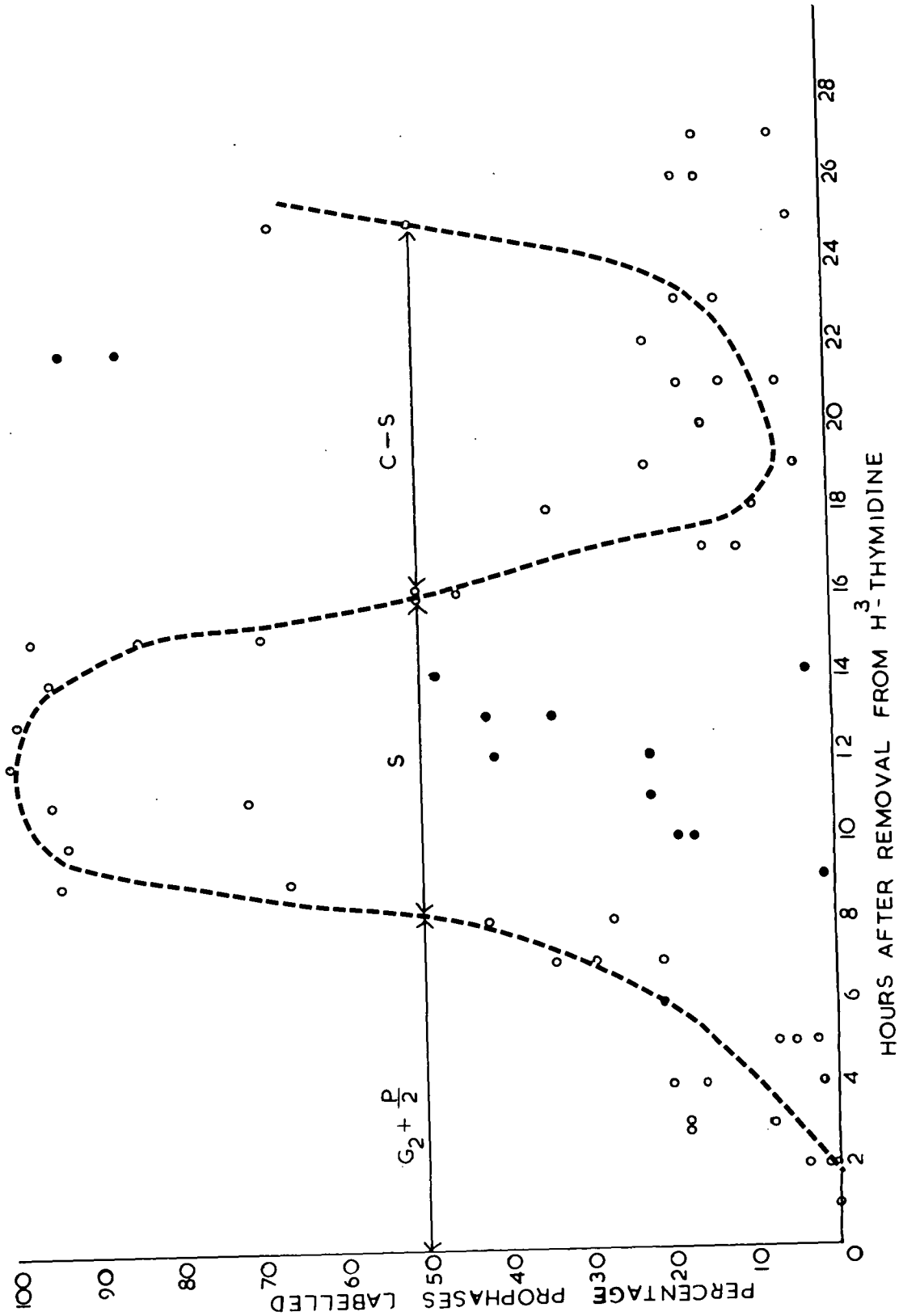


Figure 2. *Vicia faba* - The per cent labelled prophase figures of individual root tips at 22°C, at various times after a 30 min labelling period with H^3 thymidine. Solid circles - possibly aberrant root tips.

II. *Spironema fragrans*

Root tips of a single clone were used as experimental material. Cuttings were taken and their bases placed in fully aerated modified Hoagland's solution under constant light. Root tips developed from the nodes, usually giving from 2 to 10 root tips per cutting in 3 to 5 days.

Experiment C. The % labelled mitoses method was used for this preliminary experiment. H^3 thymidine at the rate of 2 µc./ml (S.A. 3.0 Cu/mM) was made available to the roots for one hour. Two root tips per fixation time were collected at hourly intervals to 18 hours. The procedure used for handling root tips was similar to that used for *Vicia faba*. Root tips were hydrolysed for 8 minutes in N HCl at 60°C. Slides were developed after four weeks. Approximately 100 mitotic cells were scored per slide. The graph for % total mitotic figures labelled versus time is shown in Figure 3. The mean value is given except for those fixation times where only 1 root tip was available; viz. 1, 3, 12, 15 and 17 hours post label.

Experiment D

In order to more fully obtain the time components of the mitotic cycle for *Spironema* the double labelling technique of Wimber and Quastler (1963) was used. This technique employs an H^3 thymidine pulse label, followed at

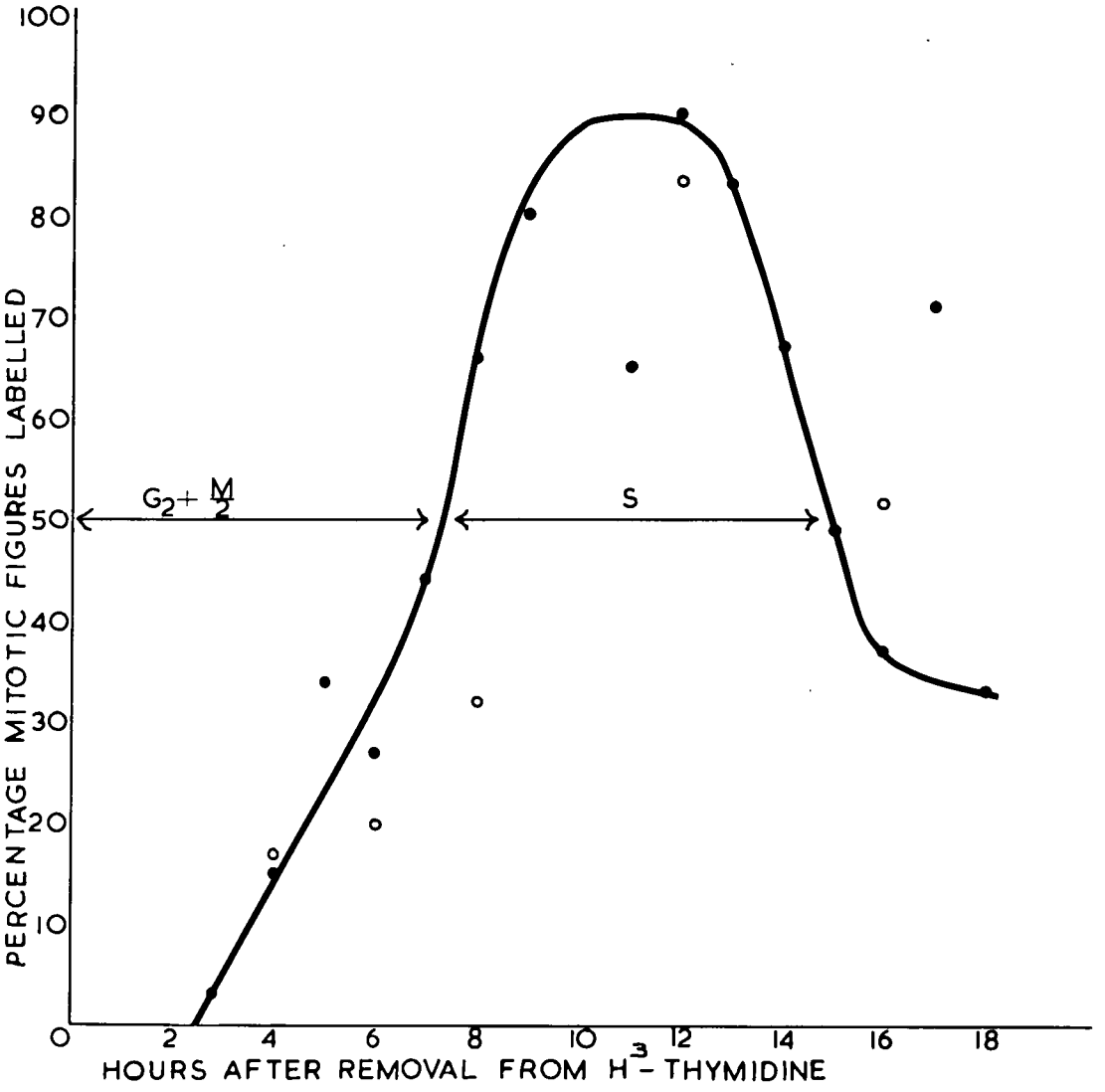


Figure 3. *Spironema fragrans* - The per cent labelled mitotic figures at various times after a one hour labelling period with H^3 thymidine. Open circles - from experiment D.

specific intervals with a pulse label of C^{14} thymidine, after which root tips are immediately fixed. Between the first and second administration of labelled thymidine a certain fraction of cells will have ceased DNA synthesis and will not be labelled by the second isotope. The fraction of cells leaving the S phase in a given interval is proportional to the ratio; length of interval/duration of S, from which S is calculated. The theory and relative equations are given in Wimber and Quastler (1963). Cells which have incorporated H^3 alone can be distinguished from those which have incorporated C^{14} since the grains in the emulsion above such cells are widely scattered and not confined to the nucleus as is the case with H^3; (H^3 β ray mean distance travelled 1 μ; C^{14} β ray mean distance travelled 50 μ; from Wimber and Quastler 1963) see Figure 4.

H^3 thymidine was made available to all rooted cuttings for 30 minutes (0.56 μc/ml; S.A. 3.0 Cu/mM). At intervals of 4, 6, 8, 12 and 16 hours post label, two cuttings were taken at random and placed in a nutrient solution containing C^{14} thymidine at 1.36 μc/ml. (S.A. 27 mc/mM) for 30 minutes. Immediately following the second label all root tips on the two cuttings were fixed. Slides were prepared as before except that after dipping in nuclear track emulsion, slides were allowed to dry

Figure 4. *Spironema fragrans* - Bright field and phase contrast figures depicting cells w with localised grains (from H^3 thymidine) and scattered grains (from C^{14} thymidine).

before being dipped a second time. The second layer of emulsion facilitates distinction of the grain scatter from C^{14} labelled cells.

All slides were scored for cells labelled with H^3 alone and cells labelled with C^{14}. C^{14} labelled cells could have had C^{14} alone or H^3 and C^{14}. Approximately 300 cells were scored per slide. Labelled and unlabelled mitotic stages were also noted during the course of this counting.

The results obtained for individual root tips are given in Figure 5 where the ratio of H^3 labelled cells to C^{14} labelled cells is shown against time between labels. The mean values for percentage mitotic figures labelled at each fixation time are shown in Figure 3. The points available are insufficient to draw any conclusions however they provide some corroboration for the results from experiment C.

Results

Although the "per cent labelled mitoses" method has been used extensively, different investigators have adopted different methods of interpretation (see Takahashi 1966 for examples). Therefore depending on the method of interpretation variable conclusions can be obtained from the same data. Takahashi (1966 p.202) has stated.

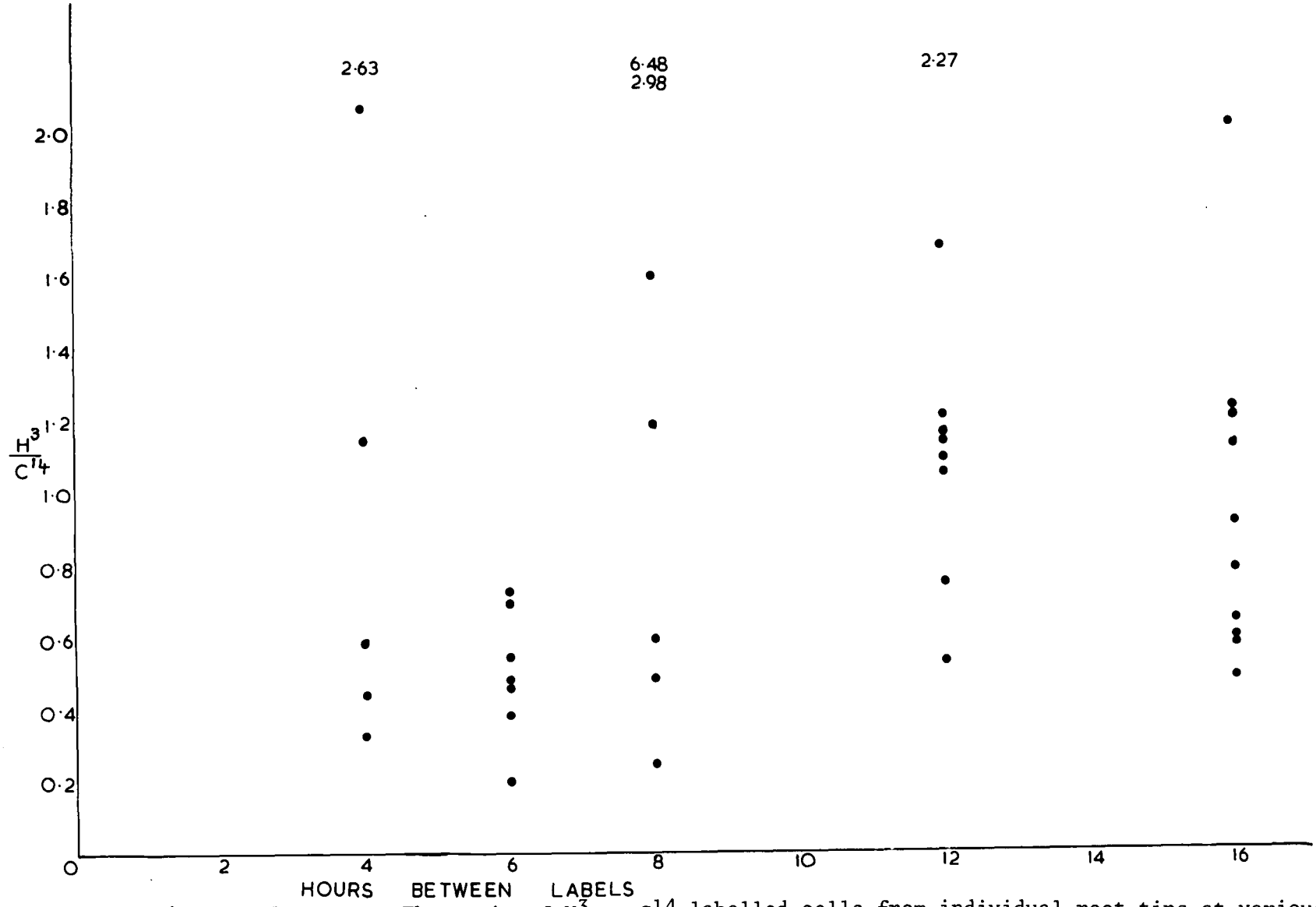


Figure 5. *Spironema fragrans*. The ratio of H^3 to C^{14} labelled cells from individual root tips at various time lapses between labelling with H^3 thymidine for 30 mins and labelling with C^{14} thymidine for 30 mins.

"Consequent differences of the estimates are sometimes beyond limits of technical accuracy, since values nearly twice as large may be yielded according to different methods". He carried out a theoretical analysis of the "labelled mitosis wave method", and concluded that the method of interpretation of Wimber (1960) was nearest to the theoretical requirements. Therefore using the method of Wimber (1960) the following values can be derived from the three % labelled mitoses experiments.

Table 1.

Mitotic cycle time parameters (hours)

	Vicia faba		*Spironema fragrans*
Experiment	A	B	C
Time from 0 to 1st 50% intercepts	(G2 + $\frac{1}{2}$ pro-met) 4	(G2 + $\frac{1}{2}$ pro) 8	(G2 + $\frac{1}{2}$ M) 7
Time from 1st to 2nd 50% intercepts = S	10	8	8
Time from 1st to 3rd 50% intercepts = C total mitotic cycle	?	17	?

The values for experiment B were obtained by considering only those root tips which responded as expected. The percentage labelled metaphases and labelled ana-telophases were also determined (not shown) and were equally as variable. However from the comparison between the idealised ascending curves a crude estimate of the duration of mitosis could be made. Since C-S = G_1 + G_2 + M; and mitosis can be estimated as 2 hours this means there is no G1 period.

Double labelling method - *Spironema fragrans*

The theory behind the double labelling method is given in Wimber and Quastler (1963). As can be seen from Figure 5 the variability between root tips was considerable. All values ≥2.0 (the theoretical limit) were not included in the calculations. The mean value for the remaining root tips at each fixation time were used, except at the 4 hour fixation time when only the lowest 3 values were averaged. This was done because these 3 root tips came from 1 cutting, whereas the other 3 root tips came from the second cutting. The results are shown in Table 2.

Table 2.

Determinations from double labelled *Spironema fragrans* root tips

Hours between treatments	H^3 labelled cells / C^{14} labelled cells ± S.E. of mean	Duration (hours)		
		S	G2+pro-meta-phase	G1+ana-telo-phase
4	0.45 ± 0.15	9(1)		
6	0.49 ± 0.02		8 (2)	
8	0.82 ± 0.13		9 (2)	- 1 (3)
12	1.07 ± 0.14			0 (4)
16	0.86 ± 0.11			- 3 (4)

The numbers in parentheses refer to the equations used; direct from Wimber and Quastler (1963, p.14).

Mitosis and the phases of mitosis can be estimated by using equation 9 (Wimber and Quastler 1963, p.18). Using the 4 hour value this results in: M 3 hours, prophase 1.5 hours, metaphase 0.5 hours, ana-telophase 1 hour.

Therefore the components of the mitotic cycle can be estimated as:

	S	G2	G1	M	C
Hours	9	6	0	3	18

Discussion

The most outstanding observation from the results is the variability between root tips. The curves that are drawn are idealised according to expectations of the method as shown by Wimber (1960). In experiments A and C variability may have been reduced if a larger sample of root tips per time interval had been taken. However it is doubtful if this would have been possible in experiment B. It is possible that those root tips represented by solid circles (Figure 2) constitute a population with entirely different cell dynamics. If a curve was drawn only considering solid circles, an estimate of G2+½ prophase of approximately 16 hours would result. If such should be the case the reason for it is unknown.

Data of other workers

The duration of the various phases of the mitotic cycle in *Vicia faba* have been estimated a number of times (see Table 3). Evans and Savage (1959) have demonstrated that temperature influences the length of the mitotic cycle in *Vicia faba*; while Wimber (1966) has shown that temperature differentially affects the length of the various phases in *Tradescantia paludosa*.

Table 3.

Duration of mitotic cycle phases in *Vicia faba*

Method	Temperature °C	Phase duration (hours)								Author
		G1	S	G2	M	C	P	M	A+T	
P^{32} labelling	19	12.0	6.0	8.0	4.0	30.0				Howard and Pelc (1953)
H^3 labelling	19 ± 0.1	4.9	7.5	4.9	2.0	19.3	1.3			Evans and Scott (1964)
H^3 labelling	19	2.37	4.0	8.0	3.36	18.0				Dewey and Howard (1963)
Colchicine metaphase accumulation	3				14.8	260	8.4	2.7	3.7	Evans and Savage (1959)
	10				6.3	64.2	4.1	0.9	1.3	
	19				3.1	26.2	1.9	0.3	0.9	
	25				1.9	22.9	1.1	0.3	0.5	
H^3 labelling	21 - 23	4	9.0	3.5	1.9	18				V'ant Hof (1967)
Colchicine metaphase accumulation		4				17-18				
H^3 labelling	18 - 21		10	<4						This study (A)
	22 ± 0.1	0	8	7	2	17	1.0	0.5	0.5	(B)

Discrepancies between studies (Table 3) are obvious. The pioneering experiment of Howard and Pelc (1959) used high doses of isotope for long periods and it is probable that a radiation induced delay in mitotic cycle time resulted. Wimber and Sparrow (1959) and Wimber and Quastler (1963) have shown that damage caused by endogenous radiation from high levels of H^3 thymidine results in mitotic delay. It is possible the long tail on the ascending curve of experiment B (Figure 2) is a consequence of radiation induced delay of cells passing through G2. However the H^3 thymidine dose does not seem sufficient to cause such a delay.

The results from the 2 *Spironema fragrans* experiments show reasonable agreement. Wimber has carried out a number of carefully conceived studies with *Tradescantia paludosa* (also in the family Commelinaceae) which also show variation even under similar conditions. (Table 4).

Variation between root tips was comparatively slight in these experiments yet under similar conditions the value for G1 varies by 4.8 hours, while there is reasonably good agreement for the other parameters. In particular the double labelling method produced variable results for G1, estimates ranging from 0 to 6 hours. (Wimber and Quastler 1963, p.17). Other workers have used the double labelling technique but only to estimate

Table 4.

Duration of mitotic cycle phases in *Tradescantia paludosa*

Method	Temperature °C	Root tips per time interval	Phase duration (hours)								Author
			G1	S	G2	M	C	P	M	A+T	
H^3 thymidine	22 ± 1	8	4.0	10.8	2.7	2.5	20.0	1.6	0.3	0.6	Wimber (1960)
Double labelling H^3 and C^{14} thymidine	21 ± 1	6	1.0	10.5	2.5	3.0	17.0	2.0		1.0	Wimber and Quastler (1963)
H^3 thymidine	21	8	5.8	10.8	2.5	1.7	20.8	1.30	0.15	0.27	Wimber (1966)

some parameters of the mitotic cycle e.g. Baserga and Lisco (1963) estimated S in Ehrlich ascites cells; Wimber and Lamerton (1963) estimated S and M in mouse intestinal cells.

Many studies utilising the percent labelled mitosis method show considerable within and between experiment variation: e.g. Evans and Scott (1964) in *Vicia faba*; Prasad and Godward (1965) in *Phalaris canariensis* and *P.minor*; Bloch *et al* (1967) in *Allium cepa*; V'ant Hof (1965, 1967) in several plants; and Ames and Mitra (1966) in *Haplopappus gracilis*. All the above being root tip experiments.

Possible sources of error

The conditions for applicability of either the per cent labelled mitoses or the double labelling methods are given by Takahashi (1966, p.208) *viz:*

1. Homogeneity of cell population.
2. Asynchrony of cell division.
3. Constancy of mean generation time.
4. Skewed distribution of phase durations.
5. Nuclear cycle not influenced by isotopic labelling (especially, no preferential death of labelled cells).
6. Practically instantaneous labelling of S phased nuclei.

If any of these factors do not hold they will contribute to variability.

1. The cellular organisation of root tips has been discussed by Clowes (1961a). In a study of *Zea mays* root tips Clowes (1961b) showed that different regions of the root tip behave quite differently from each other. Therefore the cells of the root tip as a whole have varying mitotic cycle times and are not homogeneous. Clowes (1961a) considers the *Vicia faba* root tip difficult to investigate anatomically. However he reports a clear quiescent centre in which the rate of DNA synthesis is reduced. Mattingly (1966) has reported differential regional recovery of cell division in root tips in *Vicia faba* following amino-uracil induced synchronisation. She reports (in agreement with Clowes, 1961a) that these variations in recovery time reflect differences in mitotic cycle times, existing naturally in the different tissues of the root. Dewey and Howard (1963) also propose that differentiating cells in Vicia roots move more slowly through the mitotic cycle than meristematic cells. Therefore since there are no sharp boundaries of cell types in *Vicia faba* root tips (Clowes, 1961a) they would contain an inhomogeneous population of cells.

2 and 5. For asynchrony of cell division to be present in a root tip, cells should have an equal chance of being at any point in the nuclear cycle. Since the root tip is regionalised this may not necessarily be so. Also many biological systems develop endogenous rhythms that partially synchronise cell populations. Rhythmic changes in mitotic index indicate cell synchrony. In the experiments reported, rough estimates only of mitotic index were made. They did not appear to be cyclical although they were at times very variable. Wimber and Quastler (1963) found that high levels of H^3 thymidine (4 µc/ml; S.A. 3.1 Cu/mM) produced quite different results from 1 µc/ml in *Tradescantia paludosa* root tips. The high values of H^3 labelled cells/C^{14} labelled cells they obtained were attributed to the induction of synchrony in the system by the high level of endogenous radiation. In this study some abnormally high values were obtained throughout; therefore some root tips or cuttings must have developed an endogenous rhythm before the experiment commenced. As pointed out by Wimber and Quastler (1963) the estimate of S from the first set of data in double labelling experiments is all important, since it appears in all subsequent calculations. In this case (experiment D) the estimate of S was based on the results of 3 root tips, therefore it is possibly not sufficiently precise

and this would explain the negative values obtained for G1.

Hinegardner, Rao and Feldman (1964) did not find a G1 period in rapidly developing sea urchin eggs, while Clowes (1967) found that G1 was eliminated from the mitotic cycle and DNA synthesis was advanced into telophase in the fastest dividing cells of the root tip of *Zea mays*. Alpen and Johnstone (1967) also found no G1 cells in dog bone marrow normoblasts. They reported that some cells appeared to synthesise DNA without incorporation of the labelled precursor. This may explain the aberrant results in experiment B where a few root tips at later fixation times of quite normal mitotic index showed very few labelled mitoses. To advocate these studies as corroborative evidence for the failure to find a G1 period in *Vicia* (experiment B) or *Spironema* (experiment D) would be presumptuous, however in conjunction with the extreme variability found the possibility is there.

3 and 4. Kubitschek (1962) has demonstrated a normal distribution of cell generation rate for *E.coli*, a yeast, a protozoan and He La cells. Therefore assuming no outside influences constancy of mean generation time for any particular group of cells should hold.

5 and 6. Wimber and Quastler (1963) have shown that intracellular incorporated isotopes produce sufficient endogenous radiation to influence the mitotic cycle. Since H^3 thymidine has to be made available to cells for a definite period (in this case 30 minutes or 1 hour) instantaneous labelling is impossible therefore some cellular variability is inevitable. Also intercellular variability of DNA presursors may exist such that variable degrees of dilution of the incorporated label would result.

Other factors which would also affect variability involve technical inaccuracies. Apart from straight out incompetence in carrying out the experimental method these are primarily concerned with difficulties of scoring. Cells falsely scored as negative may not have been sufficiently close to the emulsion for β ray emissions to register. Others may be rapidly dividing cells which were in G1 at the time of labelling and then moved through S and G2 in a comparatively short time. Cells may have been near the end of S when H^3 thymidine was available and did not incorporate sufficient isotope to register. Telophase cells may not have registered due to the reduction in label by one half after chromosome separation.

A H^3 labelled cell was defined (arbitrarily) as having 4 or more silver grains above it. With H^3 thymidine this is reasonable due to the limited range of

the β rays. However in the double labelling method when C^{14} thymidine is used, the cross firing from the more energetic C^{14} β rays produces many silver grains over cells adjacent to those labelled with C^{14}. Therefore an arbitrary limit for defining a cell labelled with C^{14} could not be used. This has the result that cells with a light scattering of grains were not counted. These could have been cells lightly labelled with C^{14} or cells originally labelled with H^3 and having grains above them from adjacent cells heavily labelled with C^{14}. Occasionally cells lightly labelled with C^{14} and heavily labelled with H^3 would be incorrectly classified as being labelled with H^3.

In view of the material presented and of the results given, the view of Kollmorgen, Trucco and Sacher (1967 p.49) probably understates the situation.

"The percent labelled mitoses curve is a useful method and has the advantage that it gives a good visualisation of the movement of cells through the cell cycle. However the curve is complex and it does not always yield good estimates of the duration of the component phases of the generation cycle".

Although the mitotic cycle determinations reported here are of limited value, they were adequate to

characterise general behaviour for the main part of the autoradiographic work.

REFERENCES

Alpen, E.L. and M.E. Johnstone, 1967. DNA synthetic rate and DNA content of nucleated erythroid cells. Exptl. Cell Res. *47*, 177-192.

Ames, I.H. and J. Mitra, 1966. The mitotic cycle time of *Haplopappus gracilis* root tip cells as measured with H^3 thymidine. The Nucleus *9*, 61-66.

Baserga, L. and E. Lisco, 1963. Duration of DNA synthesis in Ehrlich ascites cells as estimated by double labelling with C^{14} and H^3 thymidine and autoradiography. J. Natl. Cancer Inst. *31*, 1559-1571.

Bloch, D.P., R.A. MacQuigg, S.D. Brack and Jung-Lung Wu, 1967. The syntheses of deoxyribonucleic acid and histone in the onion root meristem. J.Cell Biol. *33:* 451-467.

Clowes, F.A.L., 1961a. Apical meristems. Blackwell Scientific Publns. Oxford.

1961b. Duration of the mitotic cycle in a meristem. J. Exptl. Botany *12*: 283-293.

1967. Synthesis of DNA during mitosis. J. Exptl. Botany *18*: 740-745.

Dewey, D.L. and A. Howard, 1963. Cell dynamics in the bean root tip. Radiation Botany *3*: 259-263.

Evans, H.J. and J.R.K. Savage, 1959. The effect of temperature on mitosis and on the action of colchicine in root meristem cells of *Vicia faba*. Exptl. Cell Res. *18*: 51-61.

Evans, H.J. and D. Scott, 1964. Influence of DNA synthesis on the production of chromatid aberrations by X-rays and maleic hydrazide in *Vicia faba*. Genetics *49*: 17-38.

Hinegardner, R.T., B. Rao and D.E. Feldman, 1964. The DNA synthetic period during early development of the sea urchin egg. Exptl. Cell Res. *36*: 53-61.

Howard, A. and S.R. Pelc, 1953. Synthesis of deoxyribonucleic acid in normal and irradiated cells and its relation to chromosome breakage. Heredity *6* (Suppl.): 261-273.

Kollmorgen, M.G., E. Trucco and G.A. Sacher, 1967. Generation cycle and analysis of Chinese hamster cells by a new method and its comparison with other methods. Exptl. Cell Res. *47*: 49-62.

Kubitschek, H.E., 1962. Normal distribution of cell generation rate. Exptl. Cell Res. *26*: 439-450.

Lima-de-Faria, A., 1959. Bibliography on autoradiography. With special reference to tritium labelled DNA precursors. Hereditas *45*: 632-648.

Mattingly, E., 1966. Differences in mitotic cycle in various tissues of *Vicia faba* roots as revealed by 5-amino uracil synchronisation of cell division. Exptl. Cell Res. *42*: 274-280.

Prasad, A.B. and M.B.E. Godward, 1965. Comparison of the developmental responses of diploid and tetraploid *Phalaris* following irradiation of the dry seed. I. Determination of mitotic cycle time, mitotic time and phase time. Radiation Botany *5:* 465-474.

Quastler, H. and F.G. Sherman, 1959. Cell population kinetics in the intestinal epithelium of the mouse. Exptl. Cell Res. *17*: 420-438.

Takahashi, M., 1966. Theoretical basis for cell cycle analysis. I. Labelled mitosis wave method. J. Theoret. Biol. *13*: 202-211.

V'ant Hof, J., 1965. Relationships between mitotic cycle duration, S period duration and the average rate of DNA synthesis in the root meristem cells of several plants. Exptl. Cell Res. *39*: 48-58.

1967. Studies on the relationships between cell population and growth kinetics of root meristems. Ibid *46*: 335-347.

Wimber, D.E., 1960. Duration of the nuclear cycle in *Tradescantia paludosa* root tips as measured with H^3 thymidine. Am. J. Botany *47*: 828-834.

1966. Duration of the nuclear cycle in *Tradescantia* root tips at three temperatures as measured with H^3 thymidine. Ibid. *53*: 21-24.

Wimber, D.E. and L.F. Lamerton, 1963. Cell population kinetics in the intestine of continuously irradiated mice, using double-labelling autoradiography. Radiation Res. *28*: 694-700.

Wimber, D.E. and H. Quastler, 1963. A C^{14} and H^3 thymidine double labelling technique in the study of cell proliferation in *Tradescantia* root tips. Exptl. Cell Res. *39*: 8-22.

Wimber, D.E. and A.H. Sparrow, 1959. Cytogenetic consequences of incorporated tritium labelled thymidine (abst.). Radiation Res. *11*: 478.